### (19) World Intellectual Property Organization International Bureau



### 

### (43) International Publication Date 21 March 2002 (21.03.2002)

### **PCT**

### (10) International Publication Number WO 02/22080 A2

(51) International Patent Classification7:

A61K

(21) International Application Number: PCT/US01/28861

(22) International Filing Date:

14 September 2001 (14.09.2001)

(25) Filing Language:

English

(26) Publication Language:

**English** 

(30) Priority Data:

60/233,180 15 September 2000 (15.09.2000)

(71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(72) Inventors; and

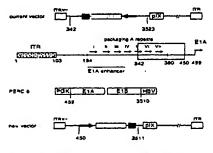
(75) Inventors/Applicants (for US only): EMINI, Emilio, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). YOUIL, Rima [AU/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). BETT, Andrew, J.

[CA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CHEN, Ling [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). KASLOW, David, C. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SHIVER, John, W. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). TONER, Timothy, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CASIMIRO, Daniel, R. [PH/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

- (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, 7.W.
- (84) Designated States (regional): ARIPO patent (GII, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian

[Continued on next page]

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS



Modifications made to the current adenovector backbone in the generation of the new

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show en-M hanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HTV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1- Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.



patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CII, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NI., PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

### Published:

 without international search report and to be republished upon receipt of that report

# TITLE OF THE INVENTION ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

### 5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2 (serial number unassigned), filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively.

10

15

20

25

30

35

## STATEMENT REGARDING FEDERALLY-SPONSORED R&D Not Applicable

### REFERENCE TO MICROFICHE APPENDIX

Not Applicable

### FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first generation adenovirus vaccines found to exhibit enhanced growth properties and greater cellular-mediated immunity as compared to other replication-deficient vectors. The invention also relates to the associated first generation adenoviral vectors described herein, which, through the incorporation of additional 5' adenovirus sequence, enhance large scale production efficiency of the recombinant, replicationdefective adenovirus described herein. Another aspect of the instant invention is the surprising discovery that the intron A portion of the human cytomegalovirus (hCMV) promoter constitutes a region of instability in adenoviral vector constructs. Removal of this region from adenoviral expression constructs results in greatly improved vector stability. Therefore, improved vectors expressing a transgene under the control of an intron A-deleted CMV promoter constitute a further aspect of this invention. These adenoviral vectors are useful for generating recombinant adenovirus vaccines against human immunodeficiency virus (HIV). In particular, the first generation adenovirus vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide pharmaceutical products, and biologically active modifications thereof. Host administration of the recombinant, replication-deficient adenovirus vaccines described herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

### BACKGROUND OF THE INVENTION

10

15

20

25

30

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5'LTR-gag-pol-env-LTR 3'organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The gag gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the pol gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The pol gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNAse H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNAse H (RNAse, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

5

10

15

20

25

30

35

The *env* gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

The *tat* gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The rev gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

5

10

15

20

25

30

35

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HTV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8<sup>+</sup> T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8+T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4<sup>+</sup> T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

5

10

15

20

25

30 .

35

European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including env or gag. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see*, *e.g.*, Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, *Nature* 327: 716-717) and Larder, et al., (1989, *Proc. Natl. Acad. Sci.* 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, FEBS Lett. 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, *J. Biol. Chem.* 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, *J. Virol*. 69: 376-386) disclose singe and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, gag, pol and nef. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

### SUMMARY OF THE INVENTION

5

10

15

20

25

30

35

The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to pol modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to nef modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH<sub>2</sub>-teriminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

5

10

15

20

25

30

35

The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Poland/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replicationdefective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication -defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5'region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced 10 growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in 15 large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use 20 in gene therapy and nucleotide-based vaccine-vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

25

30

35

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

5

10

15

20

25

30

35

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises: a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

Other aspects of this invention include a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

To this end, the present invention particularly relates to harvested recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6® cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising:

a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto, base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

5

10

15

20

25

30

35

In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

5

10

15

20

25

30

35

The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a mutlivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

10

15

20

25

30

35

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter

operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

5

10

15

20

25

30

35

It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to -- highly active antiretroviral therapy --.

"first generation" vectors are characterized as being replication-defective.

They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

"s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

15

20

25

30

35

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

"Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

"Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an <u>inactivated</u> version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

5

10

15

20

25

30

35

In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or

10

15

20

25

30

35

"MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*II site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IApol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt) is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the BgIII site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

"MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

"pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns and/orV1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

### BRIEF DESCRIPTION OF THE FIGURES

5

10

15

20

25

30

Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

5

10

15

20

25

30

35

Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

5

10

15

20

25

35

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEO ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH2-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEO ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of 30 respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "\*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

5

10

15

20

25

30

35

Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

Figure 31 shows the intracellular γIFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti-γIFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γIFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3<sup>+</sup> cells that were CD8<sup>+</sup>γIFN<sup>+</sup> and CD4<sup>+</sup>γIFN<sup>+</sup>, respectively.

Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IApol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IApol fustion frame.

5

10

15

20

25

### DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus cis-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained it correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

30

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; see, Chroboczek et al., 1992 J. Virology 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6® cell line transefected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

5

10

15

20

25

30

35

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually outcompete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

5

10

15

20

25

30

35

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on concensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized *env* sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

10

15

20

25

30

35

A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the 5 hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at 10 least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S. 15 Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an 20 amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at 25 the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs disclosed herein relate to open reading frames for cloning to the enhanced first 30 generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID 35 NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

5

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate 10 studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMVnef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-15 nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and 20 PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein 25 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef 30 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and 35 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

5

10

15

20

25

30

35

Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

5

10

15

20

25

30

35

The present invention also relates to application of a mono-, dual-, or trimodality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviralcontaining shuttle plasmids used in the construction of an adenovirus vector, this plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression regulatory elements, and a minimal pUC backbone; see Montgomery et al., 1993, DNA Cell Biol. 12:777-783. The pUC sequence permits high levels of plasmid production in E. coli and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

5

10

15

20

25

30

35

Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 pol open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine, especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

5

10

15

20

25

30

35

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly is pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possible a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+). Potential "2+1" divalent vaccines of the present invention might be a hCMV-gagbGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (e.g., nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficaceous adenovirus-based HIV-1 vaccine may be administered via a combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

10

15

20

25

30

35

Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon. Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of E. coli most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

5

10

15

20

25

30

35

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms—a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" *Advances in Pharmacology* 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed supra, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6<sup>®</sup> cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6<sup>®</sup>. Both these cell lines express the adenoviral E1 gene product. PER.C6<sup>®</sup> is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6<sup>®</sup>, from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 J. Gen. Virol 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

5

15

20

25

30

35

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM MgCl<sub>2</sub>; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM MgCl<sub>2</sub>, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface. It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of  $1x10^7$  to  $1x10^{12}$  particles and preferably about  $1x10^{10}$  to  $1x10^{11}$  particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

10

15

20

25

30

35

This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

10

15

20

25

30

35

### **EXAMPLE 1**

Removal of the Intron A Portion of the hCMV Promoter GMP grade pVIInsHIVgag was used as the starting material to amplify the hCMV promoter. PVIInsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery et al., supra for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the Msc1 site of the hCMV promoter and a 3' primer (designed to contain the BgIII recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity Taq polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with Msc1 and BglII. This fragment was then cloned back into the original GMP grade pV1JnsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following Msc1 and BgIII digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVIJnsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using BglII digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the BglII site. Colonies were screened using Sma1 restriction enzymes to identify clones that carried the Flgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)<sub>n</sub>, and (T)<sub>n</sub>; respectively) underlined:

<u>AATAAA</u>AGATCTTTATTTTCATTAGATCT<u>GTGTG TTGGTTTTTTGTGTG</u> (SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

15 EXAMPLE 2

5

10

20

25

Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: In vitro DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	μg gag/10e6 COS cells/5μg DNA/48 hr
HIVFL-gagPR9901 <sup>a</sup>	10.8
PVIIns-hCMV-FLgag-bGHpAb	16.6
pV1Ins-hCMV-FLgag-SPA <sup>b,c</sup>	12.0

<sup>&</sup>lt;sup>a</sup> GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

10

### **EXAMPLE 3**

Rodent (Balb/c) Study for Modified gag Transgenes

A rodent study was performed on the two new plasmid constructs
described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no
intron)-FLgag-SPA - in order to compare them with the construct described above
possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody
and Elispot responses (described in PCT International Application No.
PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S.
Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S.
Application Serial No. 60/148,981, filed August 13, 1999, all three applications which
are hereby incorporated by reference) were measured. The results displayed in Table
3 below, show that the new plasmid constructs behaved equivalently to the original
construct in Balb/c mice with respect to their antibody and T-cell responses at both
dosages of plasmid DNA tested, 20 µg and 200 µg.

b New plasmid constructions that have the intron A portion removed from the hCMV promoter.

<sup>&</sup>lt;sup>c</sup> In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

**EXAMPLE 4** 

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA®	Dose, ug <sup>b</sup>		Anti-p24 Titers (3 Wk PD1)°		SFC/10^6 Cells (4 Wk PD1) <sup>d</sup>					
Promoter/terminator		GMT	+SE	-SE	Media	gag197-205	p24			
HIVFL-gagPR9901	200	12800	4652	3412	2(2)	129(19)	30(11)			
(GMP grade)	20	5572	1574	1227	0	56(9)	25(6)			
pV1Jns-hCMV-	200	11143	2831	2257	0	98(5)	12(6)			
FL-gag-bGHpA	20	7352	2808	2032	0	73(9)	11(6)			
pV1Jns-hCMV-	200	16890	5815	4326	1(1)	94(4)	26(7)			
FL-gag-SPA	20	5971	5361	2825	0	85(17)	38(10)			
Naïve	0	123	50	36	0	0	0			

in PBS

5

20

Construction of the Modified Shuttle Vector - "MRKpdelE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

- (1) The left ITR region was extended to include the *Pac1* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
- 10 (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
  - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6<sup>®</sup> cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

bi.m. Injections into both quads, 50 μL per quad

<sup>°</sup>n=10;GMT, geometric mean titer; SE, standard. error

<sup>&</sup>lt;sup>d</sup>n=5, pooled spleens; mean of triplicate wells and standard. deviation. in parentheses;

#### EXAMPLE 5

# Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions ) and pADHVE3 (comprising 5 all Ad5 sequences except those nucleotides encompassing the E1 region), were each reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with Pac1 and BstZ1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either Cla1 10 linearized pAdHVO (E3- adenovector) or Cla1 linearized pAdHVE3 (E3+adenovector) into E. coli BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into E. coli XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction 15 digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple 20 cloning site of the original shuttle vector contained ClaI, BamHI, Xho I, EcoRV, HindIII, Sal I, and Bgl II sites. This MCS was replaced with a new MCS containing Not I, Cla I, EcoRV and Asc I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene. 25

# **EXAMPLE 6**

# Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the
viruses obtained from the original backbone (pAdHVE3) and the new backbone
(MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and
passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the
experiment contained the E3 gene intact and did not contain a transgene. The only
difference between the two viruses was within the region of the E1 deletion.

Following the coinfection of the viruses at P1 (passage 1), the mixtures were

propagated through an additional 4 passages at which time the cells were harvested

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac1* to remove the vector backbone) and subsequently labeled with [<sup>33</sup>P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

15

20

25

30

35

10

5

#### EXAMPLE 7

# Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following coinfection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with HindIII and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with HindIII (and Pac1 to remove the vector backbone) and then labeled with [33P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

## **EXAMPLE 8**

# 5 <u>Construction of the new shuttle vector containing modified gag transgene</u> — "MRKpdelE1-CMV(no intron)-FLgag-bGHpA"

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with Msc1 overnight and then digested with Sfi1 for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdelE1 shuttle) was linearized by digestion with EcoRV, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdelE1 shuttle vector.

20

25

30

35

15

10

# **EXAMPLE 9**

# Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdelE1-CMV(no intron)-FLgag-bGHpA, was digested with *Pac1*. The reaction mixture was digested with *BsfZ*171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with *Cla1* overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into *E. coli* BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml Terrific<sup>TM</sup> broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 μl dH<sub>2</sub>0. A 2 μl aliquot of this DNA was transformed into *E. coli* XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 μg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme *Bst*EII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

### EXAMPLE 10

# Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

30

5

10

15

20

# **EXAMPLE 11**

Virus generation of an enhanced adenoviral construct - "MRK Ad5 HIV-1gag"

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested was Pac1 to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6<sup>®</sup> cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6® cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6® cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *Hind*III and radioactively labeled with [33P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with Pac1/HindIII prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

30

5

10

15

20

# EXAMPLE 12

# Stability Analyses

5

10

15

20

25

30

35

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (in vitro gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

5

15

20

25

30

Analysis by *Hind*III digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4: Amplification Ratios Based on AEX and QPA Analysis of Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5gag	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Flgag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

5

# EXAMPLE 13

10

15

20

25

Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

<sup>\*</sup> This estimation is based on the clinical lot growth characteristics at Passage 12.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32, 905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

5

10

Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

**Table 5A:** Amplification ratios determined by AEX and QPA for **MRKAd5gag** over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

# MRKAd5gag rep1

	Xv (10 cells/n	u), Viabliny (%)	Harvest Time	Cell Passage	Titer	Tites	OPA	Ratio	Amplification	AEX
	Infection	Harvest	h.p.l.	Number	10 <sup>so</sup> vp/ml culture	10° vp/ceti	10° TC(D <sub>so</sub> /m)	AEX:QPA	Ratto	Internal Contro
P4	1.49, 81%	0.58, 50%	44	46	8.7	5.9	1.72	50	470 (MOI = 125)	
P5	1.38, 93%	0.56, 47%	48	49	6.7	4.9	1.38	49	170	
P6	1.04, 94%	0.68, 77%	47	48	5.8	5,6	1.42	41	200	•
P7	1.50, 84%	0.96, 61%	49.5	50	3.9	1.4	0.97	40	50	
P7	1.09, 97%	0.76, 59%	50	52	5.2	4.7	1.70	31	170	
P8	1.03, 94%	0.86, 64%	47.5	54	9.0	8.7	1.10	62	310	
P9	0.89, 95%	0.99, 73%	47.5	56	4,4	. 4.9	1.03	43 ·	175	3,12 2.84
P10	1.09, 91%	1.06, 66%	47,5	68	3.0	2.8	1.15	26	100	2.70 2.60
P11	1.19, 88%	0.98, 65%	47	60	3.6	9.0	1.15	31	110	2.70 2.70
P12	0.98, 91%	0.85, 63%	47,5	47	5.4	5.5	1.20	45	200	2,86 2,60
P13	1.00, 88%	0.70, 67%	49	49	5.8	5.8	1.11	62	210	3.18 3.18
P14	1.94, 92%	0.88, 67%	46	53	8.6	4.4			160	3.28 3.27
P15	0.97, 96%	0.64, 66%	47	47	6.9	7.1			250	3.12 2.91

**Table 5B:** Amplification ratios determined by AEX and QPA for **MRKHVE3** over several continuous passaging in serum free media. **MRKHVE3** is the new vector backbone which does NOT carry a transgene.

# MRKHVE3

	Xv (10° calls/m		Harvest Time	Cell Passage	Titer	Titer	QPA	Ratio	Amplification	AEX
	Infection	Harvest	h.p.i.	Number	10 <sup>10</sup> vp/ml culture	10° vp/cell	10° TCID <sub>so</sub> /ml	AEX:QPA	Ratio	Internal Control
P4	1.10, 97%	1.28, 79%	49	54	4.1	3.5	1.70	25	300 (MOI = 125)	
P5	0.92, 89%	1.18, 77%	47	. 48	4.3	4.7	1.24	35	170	
P6	1.55, 88%	1.28, 76%	49.5	50	1,2	0.8	0.56	21	30	
P6	1.09, 97%	1.11, 81%	49	52	4.0	3.6	1.18	34	130	
P7	1.17, 91%	1.22, 91%	47.5	54	3.7	8.2	0.50	74	110	
P8	0.98, 88%	1.41, 63%	46	58	2.1	2.1	0.47	45	75	3.12 2.84
Pg	1.20, 89%	1.28, 81%	47,5	58	8,0	0.7	0.29	28	25	2.70 2.60
P10	0.99, 82%	1.55, 85%	47	60	2.3	2.3	0.43	53	80	2.70 2.70
P11	1,07, 98%	1.25, 83%	48	47	2.7	2.5	0.41	66	80	2.86 2.60
P12	0.80, 91%	1.14, 60%	49.5	49	5.9	7.4	0.48	123	260	3.18 3.18
P13	1.98, 95%	1.14, 85%	45.5	53	5.8	3.0			110	3.28 3.27
P14	0.97, 96%	1.03, 98%	48,5	47	9.4	9.7			850	3.12 2.91
P15	0.87, 99%	0.97, 59%	49.5	49	5.3	6.1			218	2.78 2.52

PCT/US01/28861 WO 02/22080

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

5

15

# MRKAd5gag(E3-)

	Xv (10° pells/r Infection	ni), Viability (%) Harvest	Harvest Time h.p.l.	Cell Passage Number	Titer 10 <sup>10</sup> vp/mi culture	Tiler 10' vp/cell	QPA 10° TCID <sub>so</sub> /ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.62, 77%	1.12, 62%	47.5	48	2.0	1.2	0.92	20	100 (MOI=125)	
P5	1.16, 92%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	
P6	1.09, 97%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	180	
P7 .	1.17, 91%	0.98, 72%	47.50	54	7.1	6.1	0.67	106	220	
P8	0.98, 88%	0.77, 48%	48	56	3.1	3.2	0.66	47	115	3.12 2.84
P9	1.20, 89%	1.03, 72%	48	58	1.8	1.5	0.57	32	55	2.70 2.60
P10	0.99, 82%	0.60, 62%	46.5	60	3.2	3.2	0.68	47	115	2.70 2.70
P11	1.07, 95%	0.98, 70%	48.5	47	5.9	5.5	0.68	87	200	2.88 2.60
P12	0.80, 91%	0.87, 59%	50	49	5.1	6.4	0.72	71	230	3.18 3.18
P13	1.96, 95%	0.91, 59%	45.5	53	7.4	3.8			135	3.28 3.27
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.12 2.91
P15	0.87, 99%	0.84, 56%	49	49	4.8	5.5			196	2.78 2.52

# **EXAMPLE 14**

Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag 10 vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

## **EXAMPLE 15**

Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

20 Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (107 and 109 vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell in vitro infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this in vivo Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: In vitro analysis for gag expression in COS cells by Elisa assay.

	Viral Vectors <sup>a</sup>	
ì		

10

15

Viral Vectors <sup>a</sup>	μg gag/4.8x10e5 COS/10e8 parts/48hr
MRKAd5gag <sup>b</sup>	1.40
Clinical lot Ad5gag <sup>c</sup>	1.28
Research lot Ad5gag <sup>d</sup>	1.32
MCMVFL-gagbGHpA <sup>c</sup>	0.42

<sup>&</sup>lt;sup>a</sup> A<sub>260nm</sub> absorbance readings taken for viral particle determinations.

b MRKAd5gag was produced in serum free conditions and purified at P5.

<sup>&</sup>lt;sup>c</sup> Clinical lot# Ad5gagFN0001

<sup>25</sup> <sup>d</sup> Research Ad5FLgag lot# 6399

e mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Group ID	Vaccine	Dose (vp)	GMT	SE upper	SE lower
1	<sup>a</sup> MRKAd5gag	10^7	25600	5877	4780
2	iii Arauugag	10^9	409600	94028	76473
3	hCMV FL-gag bGHpA [E3-] →	10^7	7352	2077	1620
4	" and	10^9	235253	59767	47659
5	hCMV FL-gag SPA [E3+] →	10^7	12800	9905	236
6	•	10^9	310419	99181	75165
7	<sup>b</sup> mCMV FL-gag bGHpA [E3+] →	10^7	44572	23504	15389
8	•	10^9	941014	239068	190636
9	<sup>c</sup> hCMV FL-gag bGHpA [ <b>E3-]</b> ←	10^7	3676	934	745
10	•	10^9	117627	17491	15227
11	research lot hCMV intronA FL-gag bGHpA [E3-] <-	10^6	528	262	175
12 13	n .	10^7 10^8	14703 58813	5274 14942	3882 11915
14	•	10^9	204800	53232	42250
15	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10^6	230	82	61
18	# 55	10^7	4222	3405	1138
17		10^8	19401	3939	3274
18		10^9	89144	25187	19639
19	Naĩve	none	93	7	6

\*2x50 µL l.m. (quad) injections/animal P.l.s: Youll, Chen, Casimiro Vaccination: T. Toner, Q. Su

Assay: M. Chen

5

10

<sup>a</sup>The structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The <u>same lot</u> of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

### **EXAMPLE 16**

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses,  $10^{11}$  vp and  $10^9$  vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

<sup>&</sup>lt;sup>b</sup>The same lot of mCMVFL-gagbGHpA[E3+] used in the in vitro study (Table 6) ws used here.

<sup>&</sup>lt;sup>c</sup>This construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10e7 dose from this vector is 7 fold lower then the same dose of the MRKAd5gag and 4 fold lower than the research lot.

peripheral blood assummarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HTV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with

gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk4	Wk8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
MRKAd5gaga, 10^11 vp								
97N010	<10	118	5528	11523	7062	21997	ND	51593
97N116	<10	62	772	1447	1562	2174	ND	20029
98X007	<10	66	3353	6156	6845	3719	ND	24031
MRKAd5gag, 10^9 vp								
97N120	<10	51	204	318	366	482	ND	6550
97N]44	<10	18	118	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	ND	12851
Ad5gag <sup>b</sup> , Clinical Lot, 10^11 vp								
97X001	<10	87	2579	4718	7174	7250	Z	69226
97N146	<10	72	3604	7380	7526	18906	ZD	60283
98X009	<10	_78	4183	3946	3124	6956	ND	26226
Ad5gag, Clinical Lot, 10^9 vp								
97N020	<10	<10	143	371	390	1821	_ND	17177
97X003	<10	<10	39	93	156	596	Z	2053
98X012	<10	81	342	717	956	1558	DN	11861
MRKAc5gog (hCMV, bGHpA, E3+)								
barlginal Adagag vector (hCMV/Intra	n A bGHp	1, E3-), lot#	FN0001					
ND, not determined	, and the second second							

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4<sup>+</sup> T cells.

Grp #	Vaccination	Monkey ID	I=4	WK	Int	Wk	T=1	l Wk	I=1	6 Wk	T=2	5 Wk	T=2	8 Wk
	T=0,4,25 wks		Media	Gog H <sup>b</sup>	Media	Gog H	Media	Gog H	Media	Gog H	Media	Gog H	Media	Gog H
1	MRKA65000 10^11 vp	97N010 97N010(CD4-) 97N116 97N116(CD4-) 98X007 98X007(CD4-)	6 4 1 11 10 20	89 38 396 676 579 965	0 1 0	395 609 1304	0 3 0 0 3 0	1058 993 534 593 2193 2675	0 4 1	1174 395 2118	3 0 1 0 3	775 76 261 184 1588 1656	40000	1074 594 408 666 2113 1278
2	MRKAŒgæg 10-9 vp	97N120 97N120(CD4-) 97N144 97N144(CD4-) 98X008 98X008(CD4-)	5 11 3 6 4 14	275 170 236 148 368 696	1 8 1	249 438 1090	4 0 1 0 3	141 85 318 285 891 1175	4 3 4	119 256 673	9 0 1 ND 3	206 75 98 ND 473 391	4 1 5 0 5 4	219 219 373 625 735 848
3	AdSgog dinted let 10^11 vp	97X001 97X001(CD4-) 97N146 97N146(CD4-) 98X009 98X009(CD4-)	0 10 3 6 0	261 283 150 133 93 73	1 1 3	485 485 339	0 3 0 0 3 0	817 996 339 370 559 333	0 1 0	1220b 1272 896	1 0 3 0 1	894 1010 1238 654 384 225	0 3 0 0	1858 1123 1785 971 1748 644
4	Actigogy dinical lat 1049 vp	97N020 97N020(CD4-) 97X003 97X003(CD4-) 98X012 98X012(CD4-)	3 10 4 9 5	30 29 68 40 95 70	1 5 3	101 134 54	0 0 0 1 0	66 15 18 6 34 11	0 1 0	36 38 18	0 0 4 0 0	26 1 38 4 20 8	0 6 0 1 0	41 16 81 19 121 41
5	Ndve	96R041 053F	6 14	8 18	5	1 18.	20	0 14	D 19	0 15	0 10	0 15	1 24	9

Based on either 4x10/6 or 2x10/6 cells per well (depending on spot density)

ND, not determined

5

10

15

20

Prock or no peolide control
Pool of 20-aci peolides overlacional by 10 aci and encompassing the page sequence

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses *in vivo* even at a relatively low dose of 10^9 vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

# EXAMPLE 17 CODON OPTIMIZED HIV-1 POL AND CODON OPTIMZED HIV-1 POL MODIFICATIONS

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

on that of Hxb2r, a clonal isolate of IIIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wildtype (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize in vivo mammalian expression (Lathe, 1985, J. Mol. Biol. 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

10

15

20

25

30

35

A particular embodiment of this portion of the invention comprisies codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized))" wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC

ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

	GAAATCTGCA	CTGAGATGGA	GAAGGAGGC	AAAATCTCCA	AGATTGGCCC	CGAGAACCCC
	TACAACACCC	CTGTGTTTGC	CATCAAGAAG	AAGGACTCCA	CCAAGTGGAG	GAAGCTGGTG
	GACTTCAGGG	AGCTGAACAA	GAGGACCCAG	GACTTCTGGG	AGGTGCAGCT	GGGCATCCCC
	CACCCCGCTG	GCCTGAAGAA	GAAGAAGTCT	GTGACTGTGC	TGGATGTGGG	GGATGCCTAC
5	TTCTCTGTGC	CCCTGGATGA	GGACTTCAGG	AAGTACACTG	CCTTCACCAT	CCCCTCCATC
	AACAATGAGA	CCCCTGGCAT	CAGGTACCAG	TACAATGTGC	TGCCCCAGGG	CTGGAAGGGC
	TCCCCTGCCA	TCTTCCAGTC	CTCCATGACC	AAGATCCTGG	AGCCCTTCAG	GAAGCAGAAC
	CCTGACATTG	TGATCTACCA	GTACATGGAT	GACCTGTATG	TGGGCTCTGA	CCTGGAGATT
	GGGCAGCACA	GGACCAAGAT	TGAGGAGCTG	AGGCAGCACC	TGCTGAGGTG	GGGCCTGACC
10	ACCCCTGACA	AGAAGCACCA	GAAGGAGCCC	CCCTTCCTGT	GGATGGGCTA	TGAGCTGCAC
	CCCGACAAGT	GGACTGTGCA	GCCCATTGTG	CTGCCTGAGA	AGGACTCCTG	GACTGTGAAT
	GACATCCAGA	AGCTGGTGGG	CAAGCTGAAC	TGGGCCTCCC	AAATCTACCC	TGGCATCAAG
	GTGAGGCAGC	TGTGCAAGCT	GCTGAGGGGC	ACCAAGGCCC	TGACTGAGGT	GATCCCCCTG
	ACTGAGGAGG	CTGAGCTGGA	GCTGGCTGAG	AACAGGGAGA	TCCTGAAGGA	GCCTGTGCAT
15	GGGGTGTACT	ATGACCCCTC	CAAGGACCTG	ATTGCTGAGA	TCCAGAAGCA	GGGCCAGGGC
	CAGTGGACCT	ACCAAATCTA	CCAGGAGCCC	TTCAAGAACC	TGAAGACTGG	CAAGTATGCC
	AGGATGAGGG	GGGCCCACAC	CAATGATGTG	AAGCAGCTGA	CTGAGGCTGT	GCAGAAGATC
	ACCACTGAGT	CCATTGTGAT	CTGGGGCAAG	ACCCCCAAGT	TCAAGCTGCC	CATCCAGAAG
	GAGACCTGGG	AGACCTGGTG	GACTGAGTAC	TGGCAGGCCA	CCTGGATCCC	TGAGTGGGAG
20	TTTGTGAACA	CCCCCCCCT	GGTGAAGCTG	TGGTACCAGC	TGGAGAAGGA	GCCCATTGTG
	GGGGCTGAGA	CCTTCTATGT	GGATGGGGCT	GCCAACAGGG	AGACCAAGCT	GGGCAAGGCT
	GGCTATGTGA	CCAACAGGGG	CAGGCAGAAG	GTGGTGACCC	TGACTGACAC	CACCAACCAG
	AAGACTGAGC	TCCAGGCCAT	CTACCTGGCC	CTCCAGGACT	CTGGCCTGGA	GGTGAACATT
	GTGACTGACT	CCCAGTATGC	CCTGGGCATC	ATCCAGGCCC	AGCCTGATCA	GTCTGAGTCT
25	GAGCTGGTGA	ACCAGATCAT	TGAGCAGCTG	ATCAAGAAGG	AGAAGGTGTA	CCTGGCCTGG
	GTGCCTGCCC	ACAAGGGCAT	TGGGGGCAAT	GAGCAGGTGG	ACAAGCTGGT	GTCTGCTGGC
	ATCAGGAAGG	TGCTGTTCCT	GGATGGCATT	GACAAGGCCC	AGGATGAGCA	TGAGAAGTAC
	CACTCCAACT	GGAGGGCTAT	GGCCTCTGAC	TTCAACCTGC	CCCCTGTGGT	GGCTAAGGAG
	ATTGTGGCCT	CCTGTGACAA	GTGCCAGCTG	AAGGGGGAGG	CCATGCATGG	GCAGGTGGAC
30	TGCTCCCCTG	GCATCTGGCA	GCTGGACTGC	ACCCACCTGG	AGGGCAAGGT	GATCCTGGTG
	GCTGTGCATG	TGGCCTCCGG	CTACATTGAG	GCTGAGGTGA	TCCCTGCTGA	GACAGGCCAG
	GAGACTGCCT	ACTTCCTGCT	GAAGCTGGCT	GGCAGGTGGC	CTGTGAAGAC	CATCCACACT
	GACAATGGCT	CCAACTTCAC	TGGGGCCACA	GTGAGGGCTG	CCTGCTGGTG	GGCTGGCATC
	AAGCAGGAGT	TTGGCATCCC	CTACAACCCC	CAGTCCCAGG	GGGTGGTGGA	GTCCATGAAC
35	AAGGAGCTGA	AGAAGATCAT	TGGGCAGGTG	AGGGACCAGG	CTGAGCACCT	GAAGACAGCT
	GTGCAGATGG	CTGTGTTCAT	CCACAACTTC	AAGAGGAAGG	GGGGCATCGG	GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ
ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEO ID NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows: Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro 10 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile 15 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln 20 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val 25 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln 30 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp 35 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys 10 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val 15 Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly 20 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp 25 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to deletion of the portion of the wild type sequence encoding the protease activity, a combination of active site residue mutations are introduced which are deleterious to HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein the construct is devoid of DNA sequences encoding any PR activity, as well as containing a mutation(s) which at least partially, and preferably substantially, abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

30

DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEO ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

10

15

20

•			Table 1	
	wt aa	aa residue	mutant aa	enzyme function
	Asp	112	Ala	RT
	Asp	187	Ala	RT
30	Asp	188	Ala	RT
	Asp .	445	Ala	RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
35	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

5

10

15

20

25

30

35

To this end, SEO ID NO:3 discloses the nucleotide sequence which codes for

a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol": AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC AGGATGAGGG GGGCCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG TTTGTGAACA CCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG

CACTCCAACT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC
TGCTCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTG AGGGCAAGGT GATCCTGGTG
GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAAGGCCAG
GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGG CTGTGAAGAC CATCCACACT
GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGC CTCCATGAAC
AAGCAGGAGT AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
GTGCAGATGG CTGTGTTCAT CCACAACTCC AAGAGCAAGG GGGGCATCGG GGGCTACTCC
GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGC GAGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGGC CTCCAGGACG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID
NO:3).

5

10

15

20

25

30

35

Figure 17A-C, as follows:

In order to produce the IA-pol-based adenoviral vaccines of the present invention, inactivation of the enzymatic functions was achieved by replacing a total of nine active site residues from the enzyme subunits with alanine side-chains. As shown in Table 1, all residues that comprise the catalytic triad of the polymerase, namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues (Larder, et al., Nature 1987, 327: 716-717; Larder, et al., 1989, Proc. Natl. Acad. Sci. 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445, Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this IA Pol construct), with each residue being substituted for an Ala residue, respectively (Davies, et al., 1991, Science 252:, 88-95; Schatz, et al., 1989, FEBS Lett. 257: 311-314; Mizrahi, et al., 1990, Nucl. Acids. Res. 18: pp. 5359-5353). HIV pol integrase function was abolished through three mutations at Asp626, Asp678 and Glu714. Again, each of these residues has been substituted with an Ala residue (Wiskerchen, et al., 1995, J. Virol. 69: 376-386; Leavitt, et al., 1993, J. Biol. Chem. 268: 2113-2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene. The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys 10 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr 15 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile 20 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala 25 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys 30 · Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His 35 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations disclosed above may be suitable and therefore be utilized as an IA-pol-based adenoviral HIV vaccine of the present invention, either when administered alone or in a combined modality regime and/or a prime-boost regimen. For example, it may be possible to mutate only 2 of the 3 residues within the respective reverse transcriptase, RNase-H, and integrase coding regions while still abolishing these enzymatic activities. However, the IA-pol construct described above and disclosed as SEQ ID NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide such as is found in highly expressed mammalian proteins such as immunoglobulin leader peptides. Any functional leader peptide may be tested for efficacy. However, a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein the pol coding region or a portion thereof is operatively linked to a leader peptide, preferably a leader peptide from human tPA. In other words, a codon optimized HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. As noted in Figure 16A-B, a DNA vector which may be utilized to practice the present invention may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

10

15

20

25

30

35

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGT CTGCTGTTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA 5 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT 10 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT 15 GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT 20 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGACAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG 25 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly

Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu 10 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp 15 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile 20 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe 25 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly 30 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr.Glu.Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp 35 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

10

15

20

25

30

35

The present invention also relates to a codon optimized HIV-1 Pol mutant contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4) which comprises a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in the above paragraphs is suitable for fusion downstream of a leader peptide, such as a leader peptide including but not limited to the human tPA leader sequence. Therefore, any such leader peptide-based HIV-1 pol mutant construct may include but is not limited to a mutated DNA molecule which effectively alters the catalytic activity of the RT, RNase and/or IN region of the expressed protein, resulting in at least substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at least one point mutation which alters the active site and catalytic activity within the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion 5 junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a 10 "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows: GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA 15 GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA GCTGGGCATC CCCCACCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT 20 GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG 25 GTGGGGCCTG ACACCCCTG ACAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA 30 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA ·GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT 35 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA 10 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGCCAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA 15 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA 20 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

25 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu 30 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu 10 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr 15 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu 20 Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile 25 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val 35 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

#### **EXAMPLE 18**

## CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

10

15

20

25

30

35

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 ifrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEO ID NO:11, while the expressed open reading frame is disclosed herein as SEO ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

5

10

15

20

25

30

35

1. The nucleotide sequence of the codon optimized version of HIV-1 jrfl nef gene is disclosed herein as SEQ ID NO:9, as shown herein:

GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGGA CGGCGCCATC ACCTCCTCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC
CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
AAAGCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby incorporated by reference. See also Figure 19A-B for a comparion of wild type vs. codon optimized nucleotides comprising the open reading frame of HIV-Nef.

The open reading frame for SEQ ID NO:9 above comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine vector. The 216 amino acid HIV-1 Nef (jfrl) protein is disclosed herein as SEQ ID NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu His Pro Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

10

15

20

25

30

35

HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the inner surface of the host cell plasma membrane through myristylation of Gly-2 (Franchini et al., 1986, Virology 155: 593-599). While not all possible Nef functions have been elucidated, it has become clear that correct trafficking of Nef to the inner plasma membrane promotes viral replication by altering the host intracellular environment to facilitate the early phase of the HIV-1 life cycle and by increasing the infectivity of progeny viral particles. In one aspect of the invention regarding codon-optimized, protein-modified polypeptides, the nef-encoding region of the adenovirus vector of the present invention is modified to contain a nucleotide sequence which encodes a heterologous leader peptide such that the amino terminal region of the expressed protein will contain the leader peptide. The diversity of function that typifies eukaryotic cells depends upon the structural differentiation of their membrane boundaries. To generate and maintain these structures, proteins must be transported from their site of synthesis in the endoplasmic reticulum to predetermined destinations throughout the cell. This requires that the trafficking proteins display sorting signals that are recognized by the molecular machinery responsible for route selection located at the access points to the main trafficking pathways. Sorting decisions for most proteins need to be made only once as they traverse their biosynthetic pathways since their final destination, the cellular location at which they perform their function, becomes their permanent residence. Maintenance of intracellular integrity depends in part on the selective sorting and accurate transport of proteins to their correct destinations. Defined sequence motifs exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, Cell 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, Nature Medicine 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

10

15

20

25

30

35

Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

CATGGATGCA ATGAAGAGA GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACCCCATGTC
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCC
(SEO ID NO:11).

The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val 10 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp 15 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His 20 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12). Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 ifrl isolate wherein the codons are optimized for 25 expression in a mammalian system such as a human.

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jrfl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13, as follows:

30

GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTA ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC AGACCATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTC AAGCTGGTGC CCGTGGAGCC CGAGAAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC GCCGCCCACC CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT AAAGCCCCGGG C CCSQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

10

30

35

Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val 15 Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val 20 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His 25 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

An additional embodiment of the present invention relates to another DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGA GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG CGTGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCCATGTC CCAGCACGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCCC (SEQ ID NO:15).

10

15

30

35

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro 20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu 25 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16). An adenoviral vector of the present invention may comprise a DNA sequence, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion of substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

20 EXAMPLE 19

10

15

25

30

35

#### MRKAd5Pol Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BgIII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 (or MRKpAdHVE3) preplasmid. The vector, similar to the original shuttle vector contains the Pac1 site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with BgI II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the BgIII site. The clones were checked for the correct orientation of the gene by using restriction enzymes DraIII/Not1. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FLpol+bGHpA(S) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)Cla1. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FLpol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

10

15

20

25

30

35

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12  $\mu$ g of pMRKAd5pol was digested with restriction enzyme PacI (New England Biolabs) and 3.3  $\mu$ g was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc.). PacI digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6® cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at  $\leq$  -60°C. This pol containing recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

#### **EXAMPLE 20**

## MRKAd5Nef Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector

MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac1* site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl11* site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl11* releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the

10

15

20

25

30

35

MRKpdelE1+CMVmin+BGHpA(str.) shuttle vector at the *Bgl*11 site. The clones were checked for correction orientation of the gene by using restriction enzyme *Sca*1. A positive clone was isolated and named MRKpdelE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdelE1hCMVminFL-nefBGHpA(s) was digested with restriction enzymes *Pac*1 and *Bst*1107 I (or its isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*1 digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdelE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6<sup>®</sup> adherent monolayer cell culture. To rescue infectious virus, 12 μg of pMRKAdnef was digested with restriction enzyme *Pac1* (New England Biolabs) and 3.3 μg was transfected per 6 cm dish of PER.C6<sup>®</sup> cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech

of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

Inc.). Pac1 digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6®cells. Infected cells and media were harvested 6-10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at  $\leq$  -60°C. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

## **EXAMPLE 21**

5

10

15

20

25

30

35

Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (Not I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (Bgl II)Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the Not I and the  $Bgl \coprod$  sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with Not I and Bgl II. The mCMV promoter (Not I/Bgl II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with Bgl II and the gag reporter gene (Bgl II fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (Asc I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (Bgl II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the Asc I and Bgl II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with Asc1 and Bgl11 to remove the hCMV-gag portion of the transgene. The mCMV promoter (Asc1/Bgl11 digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with Bgl11 and the gag reporter gene (Bgl11 fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

 $Bgl ext{ II}$  site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by  $Bgl ext{ II}$  digestion.

5

10

15

35

#### **EXAMPLE 22**

Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. Pac1 and BstZ110I digestion of each shuttle vector was performed and each specific transgene fragment containing the flanking Ad5 sequences was isolated and co-transformed with Cla I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 E. coli cells. Recombinant preplasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently prepared in large scale following transformation into XL-1 Blue E. coli cells and analyzed by restriction analysis and sequencing.

### **EXAMPLE 23**

Construction of hCMV-tpa-nef (LLAA) Adenovector

The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with BamHI, gel purified and cloned into the Bgl II site of MRKAd5CMV-bGHpA shuttle vector (Bgl II digested and calf intestinal phosphatase treated). Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following Sca I digestion. The resulting MRKAd5tpanef shuttle vector was digested with Pac I and Bst Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial homologous recombination techniques.

## **EXAMPLE 24**

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c

mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol

(E3+) at either 10^7 vp and 10^9 vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively. For all rodent immunizations, the Ad5 vectors were diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl2, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50 µL aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively.

Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10^9 vp and 10^11 vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either 10^9 vp and 10^11 vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.005% polysorbate 80, pH 8.0) into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester; NY) were coated by overnight incubation with 100 μL of 1 μg/mL HIV-1 RT protein (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 uL of 1 ug/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Hunstville, AL) and incubated for 2 h with 200 μL/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was performed followed by 4-fold serial dilution. 100-μL aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100  $\mu$ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100  $\mu$ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100  $\mu$ L of 0.5M H<sub>2</sub>SO4 per well. OD<sub>492</sub> readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD<sub>492</sub> (2.5 times the background value).

Non-human primate and murine ELIspot assays - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INFγ-secreting cells from mouse spleens (Miyahira, et al.1995, J. Immunol. Methods 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at 5x10<sup>6</sup>/mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM β-ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, Current Protocols in Immunology. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100 μL/well of either 5 μg/mL purified rat anti-mouse IFN-γ IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15 ug/mL mouse anti-human IFN-γ IgG2a (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200 μL/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 μL of cell samples (4-5x10<sup>5</sup> cells per well) and 50 μL of the antigen solution were added. To the control well, 50 μL of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 ug/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4<sup>+</sup>-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8<sup>+</sup>-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8<sup>+</sup> T cell epitope) or aa81-100 (CD4<sup>+</sup>) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO<sub>2</sub>, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 μL/well of either 1.25 μg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 ug/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 μL/well 1/2500 dilution of strepavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 μL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10<sup>6</sup> cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 uL of each sample is incubated with 15 uL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN<sub>3</sub>) and 20 uL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 uL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10<sup>4</sup>7 vp. The humoral responses are highly dosedependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

				1A	ti-RT IgG Tite	rs*	8	FC/10^6 cell	s°
Group	Vaccine	Dose	No. of Doses	GMT	+SE	-SE	Medium	CD4+ peptide pool	CD8+ peptide
1	MRKAdShCMVFLpol (E3+)	10^7 vp	2 1	310419 919	301785 372	153020 265	1(1) 1(1)	75(4) 72(9)	2313(67) 533(41)
2	MRKAd5hCMVFLpol (E3+)	10^9 vp	2	1638400 <sup>b</sup> 713155	0 528520	0 303555	2(2) 1(1)	114(9) 48(7)	2083(182) 733(89)
3	MRKAd5hCMVFLpol (E3-)	10^7 vp	2	310419 6400	386218 14013	172097 4393	0(0) 10(8)	223(7) 141(21)	2807(27) 409(28)
4	MRKAd5hCMVFLpol (E3-)	10 <b>^9</b> vp	2	1638400 <sup>b</sup> 1241675 <sup>b</sup>	0 396725	0 300661	1(1) 0(0)	160(13) 39(13)	2385(11) 833(83)
Б	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)

<sup>\*</sup>GMT, geometric mean liter of the cohort of 5 mice; SE, standard error of the gemetric mean

5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and(3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

				Az	ti-nef IgG Tite	ers"	S	FC/10^6 cell	sb
Group	Vaccine	Dose	No. of Doses	GMT	+SE	-SE	Medlum	aa51-70 CD8+	aa81-100 CD4+
1	MRKAdShCMVFLnef (E3+)	10^7 vp	2 1	174 132	70 42	50 32	1(1) 0(0)	23(1) 0(0)	1(1) 0(0)
2	MRKAdShCMVFLnef (E3+)	10^9 vp	2	174 132	70 42	50 32	0(0) 1(1)	61(7) 62(7)	4(2) 3(1)
3	MRKAd5mCMVFLnef (E3+)	10^7 vp	2	132 115	42 48	32 33	3(1) 3(2)	15(5) 3(2)	5(2) 4(2)
4	MRKAd5mCMVFLnef (E3+)	10^9 vp	2 1	132 132	42 42	32 32	4(2) 2(1)	83(13) 29(2)	5(1) 4(0)
5	MRKAd5mCMVtpanef(E3+)	10^7 vp	2	132 100	42 0	32 0	3(2) 3(1)	14(2) 13(4)	5(1) 10(3)
6	MRKAd5mCMVtpanef(E3+)	10^9 vp	2	230 115	170 46	98 33	3(2) 7(1)	145(29) 151(14)	4(0) 10(0)
7	Naïve	none	none	152	78	52 ·	21(2)	· 18(6)	26(3)

<sup>\*</sup>GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean

15

Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

Near or at the upper limit of the serial dilution; hence, could be greater than this value

<sup>&</sup>lt;sup>e</sup>No. of Spot-forming Cells per million spiecnoytes; mean values of triplicates are reported along with standard errors in parenthesis.

No. of spot-forming cells per million splecnoytes; mean values of triplicates are reported along with standard errors in parenthesis.

peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus

10 Macaques.

Vaccine (T=0,4 wks)	Monk #		Prebleec	1		T=4			T=7			T¤16	
		Mock	PolL	Pal R	Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R
MRKAd5hCMV-IApol(E3+)	99C100	1	0	0	1	38	31	0	52	146	0	49	715
10^11 vp	99C215	1	2	2	10	98	249	1	109	305	22	88	250
	99D201	5	5	4	6	149	95	0	40	35	0	35	18
MRKAd5hCMV-IApol(E3+)	99D212	0	2	0	4	331	114	0	58	14	0	6	6
10/9 Vp	99D180	0	4	2	0	19	192	4	36	156	5	38	108
	99C201	8	5	21	6	62	82	0	18	32	ן י	14	65
MRKAc5hCMV-IApol(E3-)	99D239	5	2	2	20	82	172	1	68	114	9	21	40
10^11 vp	99C186	4	12	6	5	120	421	2	271	489	16	875	530
	99C084	1	8	8	8	84	484	0	14	236	ו	24	264
MRKAd5hCMV-IApal(E3-)	CC7C	10	10	8	12	724	745	4	322	376	4	188	176
10/9 vp	ထၢေ	2	0	1	5	474	468	0	232	212	0	101	121
	<b>CD11</b>	6	6	12	10	98	110	5	60	80	8	25	34
Nove	083Q	nd	nd	nd	nd	nd	nd	4	2	2	2	1_	2

nd, not determined Reported are SFC per million PBMCs; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

T =4	T =7	T=12	T=16
61	1999	5928	4768
81	1541	2356	2767
53	336	539	387
10	40	49	68
<10	36	_79	93
<10	37	71	76
44	460	1234	1015
21	233	480	345
235	2637	2858	1626
32	175	306	235
20	140	273	419
15	112	149	237
	61 81 53 10 <10 <10 210 235	T=4 T=7  61 1999 81 1541 53 336  10 40 <10 36 <10 37  44 460 21 233 235 2637  32 175 20 140	T=4         T=7         T=12           61         1999         5928           81         1541         2356           53         336         539           10         40         49           <10

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

5

15

20

25

10 Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk #	P	re	T:	=4	T:	<b>=7</b>	T=	:16
		Mock	Nef	Mock	Nef	Mock	Net	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CD2D	0	4	31	440	4	368	1	251
10^11 vp	CC7B	0	0	2	521	٥	178	1 1	1522
<u> </u>	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CC2K	9	9	6	52	0	35	0	15
10^9 vp	CD15	5	4	30	998	2	586	0	434
	CD16	6	1	6	1146	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D191	1	5	4	614	0	298	2	419
10^11 vp	99D144	4	6	5	434	0	1100	2	932
·	99C193	1 1	2	1	58	1	22	0	64
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D224	1	11	14	231	1	125	0	70
10^9 vp	99D250	8	9	4	108	0	54	0	5
	99C120	1	6	20	299	0	92	0	79
Naive	083Q	nd	nd	18	22	4	5	2	1

#### **EXAMPLE 25**

Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nef-b) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were about 85% of the clade B counterpart (Figure 25). These results suggest that cellular immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapetic advantage on a global scale.

Table 15
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

5

10

20

subject	bleed date	gag epitope #	mock	gag H-b	gagH-c	nef-b	nef-c
<b>*</b>		from mapping)					
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99	4	5	1055	1080	2210	2140
	1						

EXAMPLE 26

Characterization and Production of MRKAd5pol and MRKAd5nef

Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5

seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer (10 <sup>10</sup> vp/ml culture)	AEX Titer (10 <sup>4</sup> vp/cell)	Amplification Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

Roller Bottle Passaging - Passaging of the pol and nef constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (tritonlysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

			of cells/ml), ity (%) Harvest	Cell Passage Number	AEX Titer (Cell Associated) 1010 vp/ml culture	Titer 10' vp/cell	Amplification  Ratio	Triton Lysis Titer  10 <sup>10</sup> vp/ml culture
hCMV-FL-nef [B3+]	pool	1.22, 85%		62	0.8	0.7	25	1.6
	1		0.99, 62%					· · · · · · · · · · · · · · · · · · ·
	2		1.10, 72%		}			
hCMV-FL-pol (E3+)	pool	1.42, 89%		62	4.5	3.2	115	7.0
	1		1.22, 70%					
	2		1.42, 74%					

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

		Visbil	0° cells/ml), ity (%)	Cell Passage	AEX Titer (Cell Associated)	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	1010 vp/ml culture	10 <sup>4</sup> vp/cell	Ratio	10 to vp/ml culture
hCMV-FL-nof [E3+]	Pool	1.33, 90%		66	1.0	0.8	29	2.1
	1		0.96, 70%					
	2		1.18,73% .					
bCMV-FL-pol [B3+]	Pool	0.90*, 90%		56	4.2	4.7	168	6.5
			1.18, 88%					
_	2		1.04, 80%					

MRKAd5nef and MRKAd5pol Viral Production Kinetics - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of

20 MRKAd5gag. PER.C6® cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

Comparison of hCMV- and mCMV-FL-nef - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6® cells- experiments are underway at V&CB to measure nef expression levels.

15

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

	[	Xv (10 <sup>6</sup> cells/m	l), Viability (%)	Cell Passage	AEX Titer	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10 <sup>10</sup> vp/ml culture	104 vp/cell	Ratio	10 <sup>10</sup> vp/ml culture
hCMV-FL-nef	Pool	1.11, 91%		60	1.5	1.4	50	2.8
(MRKAd5nef)	1		1.23, 75%					
	2		1.34, 74%					
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
}	1		1.49, 84%					
	2		1.18, 77%					

20

25

#### EXAMPLE 27

Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

Materials and Methods - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6® cells at a concentration of 0.2x106 cells/ml. Cells were grown until they reached a cell concentration of approximately 1x106 cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 °C	
DO	30%	
PH	7.30	
Agitation	150 rpm	
Sparging	None	•

Table 21: Virus source used for experiments.

10

15

5

Run	Batch ID	Cloned/Uncloned	MOI
		MRKAd5nef	(vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
	B20010202-2	Cloned	280

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

Table 22: Virus Concentration as measured by the AEX assay

Run	Batch ID	Cloned/Uncloned	V	irus Concentration (	9 48hpi (1x)	10 <sup>13</sup> vp/L)
		MRKAd5nef	Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76
	B20010115-2	Uncloned	0.38	1.67	2.05	2.46
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88
	B20010202-2	Cloned	0.50	6.00	6.50	8.47

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Uncloned		Virus Concent	ration @ 48hpi	i (1x10 <sup>11</sup> IU/L)	
		MRKAd5nef	Whole	Supernatant	Clarified	Total	Triton
		j	Broth		Lysate		Lysate
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28
	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89
	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47

20

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

# EXAMPLE 28 MRKAd5HIV-1gag Boosting of DNA-Primed Animals

5

10

15

20

25

30

Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pVIJnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10e7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10e7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, CD4<sup>+</sup>-biased or CD8<sup>+</sup>-biased, and (b) boosting with the MRKAd5gag construct produced in all cases a strongly CD8<sup>+</sup>-biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific CD8<sup>+</sup> T cells.

Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkeys with MRKAd5gag Number of SFC/million PBMCs

ģ	Priming	Boost	Honk#	T=0	C	P=1		1	Te6	T=10	2	۲	1:17	T=24	z	Te28	- -	T=30	
	T=0, 4, 8 wrks	T=26 w/ks		Medium	gap H	Medium	Dag H	Медіст		Medium	H DED	Medium	H GAD	Wedium	H 08B	Wedlun	H DED	Medium	H Dan
-	DNA5 mgs	MFKAdSgag(E3+)	H985	Ş	AA	3	35	15	14	-	727	-	135	6	133	5	888		316
	SB4	10^7 vp	X800	•	0	0	5	•	46	0	89	0	7.5	0	ĸ	(7)	1705	_	755
	(D101)		AW3G	2	=	0	88	6	5	60	46	N	8	60	88	2	686	•	335
~	DNA5mga +	MFVAd5gag(E3+)	<u> </u>	0	*	-	8	0	111	9	220	4	280	8	232	5	658	9	1345
	CRL1005/45mgs .	10~7 vp	S S	7	0	-	101	0	254	0	<u>8</u>	ທ	\$	0	8	•	1915	-	1099
		1	AWap	6	0	-	₽	~	=	4	15	60	Ş	10	8	=	88	9	241
			CBSF	Š	ž	0	ਨ	0	88	0	8	6	374	60	251	80	1549	8	734
			AKBB	on .	12	4	8	-	119	•	£39	0	52	0	316	4	1229	ı,	456
3	DNA/5 mgs+	MP#(AdSoan(E3+)	AW20	٥	4	ŀ	82	147	264	61	502	9	Ē	٠	206	ă	FRS	۵	2
	CRL 1005/7.5 mgs + 0.6 mM BAK	10^7 vp	CAR	-	0	6	121	_	55	-	270	· co	8	-	5	7	1384	. 0	878
			CBSB	8	9	0	9	6	119	•	27.4	60	28	-	208	0	92		828
			CBSW	4	8	•	8	-	91	Ö	139	0	20	,	8	LC)	3	-	348
	-		CB7D	-	0	•	136	•	316	-	8	ю	929	-	759		872	7	183
4	none	None	960201	100	٥	٥	0	-	٥	٥	°	0	-	-	2	6	•	-	-
NA NA	and assembly																		

## **EXAMPLE 29**

## Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the IA pol gene (consisting of RT, RNAseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNAse H and integrase (1350 amino acids; SEQ ID NO: 39).

5

10

15

20

25

30

35

The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IApol to a unique BamHI site. This fragment was digested with BstEII and BamHI. Construction of gag-IApol fusion was achieved via three-fragment ligation involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII fragment of the V1R-gagpol containing the entire ORF of gag-IApol fusion gene.

#### **EXAMPLE 30**

Immunogenicity Studies in Non-Human Primates

Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized

5 HIV-1 gag, pol, gagpol, nef in rhesus macaques

Grp#	gag, poi, gagpoi, nei in rhesus mac	Monk #			T=6 wks		
	T=0, 4 wks		Mock	Gag H	Pol - 1	Pol - 2	Net
1	MRKAd5 gag	CB9V	0	15			-
1	10^10 vp	CD19	ο.	374		-	-
		109H	1	843	-	•	-
2	MRKAd5 gag	99D130	1	948	•	-	-
	10^8 vp	W277	16	324	-	-	-
		143H	4	595	•	-	•
3	MRKAd5 pol	CC1X	4	-	46	256	-
	10^10 vp	WEWA	3	-	463	550	-
	•	AV43	6	-	95	1333	-
4	MRKAd5 pol	AW38	1	-	19	30	-
	10^8 vp	CC8K	0	-	50	995	-
	,	CC21	1	-	33	· 436	-
5	MRKAd5 nef	076Q	9	-	-	-	1204
_	10^10 vp	091Q	4		-	-	85
	·	083Q	0	-	-	-	176
6	MRKAd5 nef	00C029	1	-	-	-	114
	10^8 vp	98D022	6	-	-	-	170
		98D160	3	-	-	-	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D251	3	206	15	193	120
	10^10 vp each	05H	3	135	21	9	638
		00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D215	1	171	18	193	240
	10^8 vp each	81H	5	73	6	14	243
		12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef	99D211	0	83	56	838	725
	10^10 vp each	22H	4	385	119	1194	1915
		61H	4	343	11	765	853
10	MRKAd5gagpol +MRKAd5 nef	34H	3	78	19	5	75
	10^8 vp each	48H	1	65	105	46	43
		70H	5	158	15	220	191
	1 0 . C . 31 '11' DT			., ,	<del>,,,,</del>		

Indicated are numbers of spot-forming cells per million PBMCS against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10^6 PBMC.

## WHAT IS CLAIMED IS

5

10

A recombinant adenoviral vaccine vector at least partially deleted in

E1 and devoid of E1 activity, comprising:

- a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to between from about base pair 400 to about base pair 458 of a wildtype adenovirus genome; and
- b) a gene encoding an HTV protein or immunologically relevant modification thereof.
- A vector in accordance with claim 1 comprising a packaging region corresponding to from about base pair 1 to about base pair 450 of a wildtype adenovirus genome.
- 3. A vector in accordance with claim 1 further comprising nucleotides
   15 corresponding to between from about base pair 3511 to about 3524 to about base pair
   5798 of a wildtype adenovirus genome.
  - 4. A vector in accordance with claim 3 comprising base pairs corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
- 5. A vector in accordance with claim 4 which is deleted of base pairs451-3510.
  - 6. A vector in accordance with claim 1 which is at least partially deleted in E3.
  - 7. A vector in accordance with claim 6 wherein the E3 deleted region is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

- 9. A vector in accordance with claim 1 wherein the vector comprises a gene expression cassette comprising:
  - a) a nucleic acid encoding a protein;

5

10

- b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and
  - (c) a transcription termination sequence.
- 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.
  - 11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation
- 12. An adenoviral vector in accordance with claim 9 wherein the geneexpression cassette is in an E1 antiparallel orientation.
  - 13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
  - 14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.
  - 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.
    - 16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

- 18. A cell comprising the adenoviral vector of claim 1.
- 19. Recombinant, replication-defective adenovirus particles harvested
   and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell
   line which expresses adenovirus E1 protein at complementing levels.
  - 20. An HTV vaccine composition comprising purified adenovirus particles of claim 19.
- 21. An HIV vaccine composition of claim 20 which comprises aphysiologically acceptable carrier.
  - 22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
  - 23. A method according to claim 22 wherein the cell is a PER.C6<sup>®</sup> cell.

15

- 24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.
- 25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

- 27. A method according to claim 24 wherein the adenovirus vaccine is
  5 preceded by an adenovirus vaccine of a different serotype.
  - 28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.
  - 29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.
- 30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.
  - 31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
- a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
  - b) a gene expression cassette comprising
    - i) SEQ ID NO: 29;
    - ii) a heterologous promoter operatively linked to i); and
    - iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

- 33 An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

5

10

15

- 35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.
  - 37. A cell comprising the adenoviral vector of claim 30.
  - 38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell line which expresses adenovirus E1 protein at complementing levels.
  - 39. An HTV vaccine composition comprising purified adenovirus particles of claim 38.
  - 40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.
- 41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6® cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.

- 44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
- 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.
  - 46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
- 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.
  - 48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.
- 49. An adenoviral vector in accordance with claim 9 wherein the gene
   20 expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.
  - 50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

5

- b) a gene expression cassette comprising
  - a nucleotide sequence selected the group consisting of
     SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
  - ii) a heterologous promoter operatively linked to i); and
  - iii) a transcription termination sequence.

10

- 51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.
- 52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.

15

- 53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

- 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.
  - 56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

- 58. An HTV vaccine composition comprising purified adenovirus particles of claim 57.
  - 59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.
  - 60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

10

- 61. A method according to claim 60 wherein the cell is a PER.C6® cell.
- 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.
  - 63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
  - 64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

- 66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 5 67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.
  - 68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.
  - 69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

10

15

- a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- b) a gene expression cassette comprising
  - i) a nucleotide sequence selected the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15;
  - ii) a heterologous promoter operatively linked to i); and
  - iii) a transcription termination sequence.
- 70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

- 72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
  - 74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.
    - 75. A cell comprising the adenoviral vector of claim 68.

10

15

- 76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.
- 77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.
  - 78. An HTV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.
  - 79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
  - 80. A method according to claim 79 wherein the cell is a PER.C6® cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

- 82. A method according to claim 81 which further comprises

  administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
- 83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.
  - 84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
  - 85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.

15

- 86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:
  - a) gag, pol, and nef, expressed independently from three individual vectors;

5

)

5

)

b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences; c) gag, pol, and nef, expressed via two vectors, one expressing a polnef fusion, and another expressing gag; d) gag, pol, and nef, expressed via two vectors, one expressing a gagpol fusion and another expressing nef; e) gag, pol and nef, expressed via two vectors, one expressing a nefgag fusion and another expressing pol; f) gag, pol, and nef, expressed via one vector expressing a gag-pol-1( nef fusion; g) gag and pol, expressed independently from two individual vectors; h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences; 15 i) pol and nef, expressed independently from two individual vectors; j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences; k) nef and gag, expressed independently from two individual vectors; 20 1) nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct

promoters and transcription termination sequences;

m) gag and pol, expressed via one vector expressing a gag-pol fusion;

n) pol and nef, expressed via one vector expressing a pol-nef fusion; and

- o) nef and gag, expressed via one vector expressing a nef-gag fusion.
- 87. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.

- 88. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the fused sequences have the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences.
- 89. A multivalent adenovirus vaccine composition in accordance with
  10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences
  operatively linked to a single promoter; and the encoding nucleic acid sequences
  operatively linked by an internal ribosome entry sequence ("IRES").

## Original Adenovector Construct:

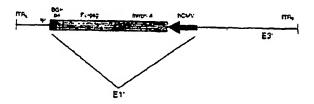


Figure 1: Original HIV-1 gag adenovector.

## Sequence of the open reading frame for FL-gag (human codon optimized)

atgggtgctagggcttctgtgctgtctggtggtgagctggacaagtgggagaagatcaggctgaggcctggtgg caagaagaagtacaagctaaagcacattgtgtgggcctccagggagctggagaggtttgctgtgaaccctggc agctgaggtccctgtacaacacagtggctaccctgtactgtgtgcaccagaagattgatgtgaaggacaccaag gaggccctggagaagattgaggaggagcagaacaagtccaagaagaaggcccagcaggctgctgctggc acaggcaactccagccaggtgtcccagaactaccccattgtgcagaacctccagggccagatggtgcaccag gccatctcccccggaccctgaatgcctgggtgaaggtggtggaggagaaggccttctcccctgaggtgatccc catgitctctgccctgtctgagggtgccacccccaggacctgaacaccatgctgaacacagtgggggggccatc aggetgecatgeagatgetgaaggagaceateaatgaggaggetgetgagtgggacaggetgeateetgtge acgctggccccattgcccccggccagatgagggagcccaggggctctgacattgctggcaccacctccaccct ccaggagcagattggctggatgaccaaccaccccccatccctgtgggggaaatctacaagaggtggatcat cccttcagggactatgtggacaggttctacaagaccctgagggctgagcaggcctcccaggaggtgaagaact ggatgacagagaccctgctggtgcagaatgccaaccctgactgcaagaccatcctgaaggccctgggccctg ctgccaccctggaggagatgatgacagcctgccagggggtggggggccctggtcacaaggccagggtgctg gctgaggccatgtcccaggtgaccaactccgccaccatcatgatgcagagggcaacttcaggaaccagag gaagacagtgaagtgcttcaactgtggcaaggtgggccacattgccaagaactgtagggcccccaggaaga agggetgetggaagtgtggcaaggaggccaccagatgaaggactgcaatgagaggcaaggccaacttcctg ggcaaatctggccctcccacaagggcaggcctggcaacttcctccagtccaggcctgagcccacagcccct agetglaecceetggeeteetgaggteetgtttggeaacgacceeteeteecagtaaaataaageeegggea gat (SEQ ID NO: 29)

Figure 2

### Old Transgene:



### New Transgenes:

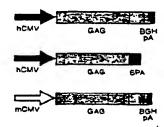


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

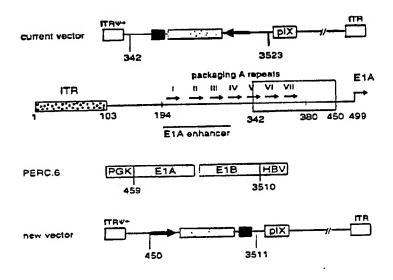


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

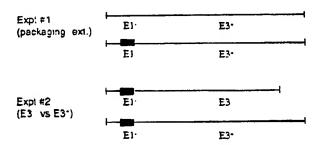


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.

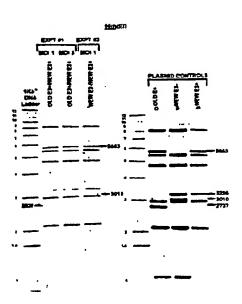


Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

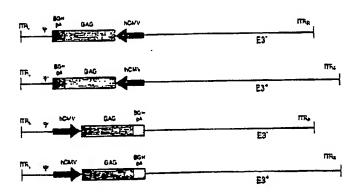


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

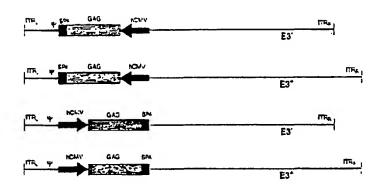


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

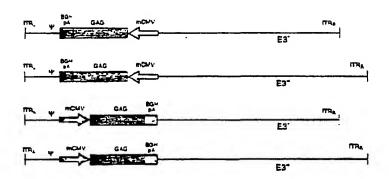


Figure 7C: mCMV-FLgag-bGHpA adenovectors constructed within the \*MRK\* backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

## Plasmid mixing expt: (orientation)

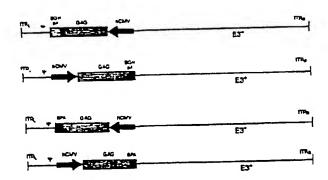


Figure 8A: Effect of transgene orientation

### Plasmid Mixing expt: (poly A signal)

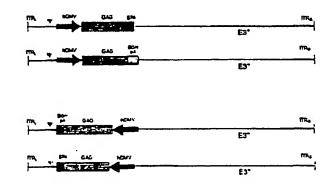


Figure 8B: Effect of polyadenylation signal

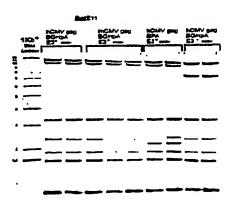


Figure 9: Viral DNA from the four Adgag candidates at P5, following BsfE11 digestion.

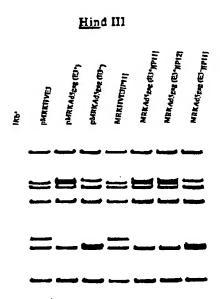


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

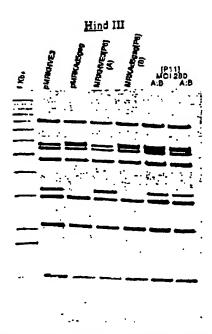


Figure 11: Viral DNA analysis (*Hind*III digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).

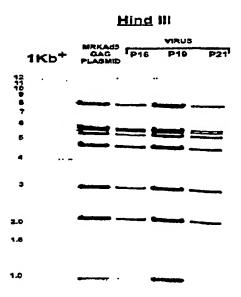
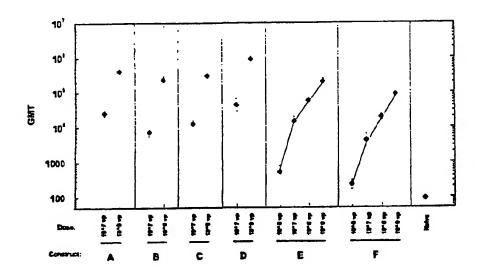


Figure 12: Viral DNA analysis by *HindIII* digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *HindIII*), and MRKAd5gag virus continually passaged to P16, P19 and P21(serum containing media).

Figure . Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb'c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5): (B) MRKAd5 E3' hCMV-FLgag-bGHpA; (C) MRKAd5 E3' hCMV-FLgag-SPA; (D) MRKAd5 E3' mCMV-FLgag-bGHpA; (D) research Lot (293 cell-derived) of Ad5HIV-lgag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-lgag. Reported are the geometric mean titers (GMT) for each cohort.



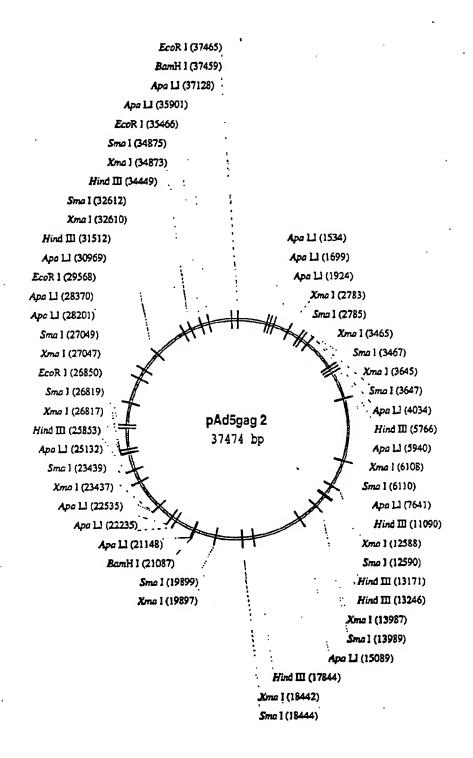


Figure 14

ACCAGANI PA TRACATETAE CATGGGTGET AGGGCTTETG TGETGTETGG GUTTAGAGAGG CAGCTGAGGT GGTCTACCAC. TCATGTAGAT CCATICKGCAC GACAAACTO GAGGTATCTT CTGTGGCCC" CCTATACTAI CATTAGETICA GTAATCAAGT TGACGICAAT AATGACGIAT ACTOCAGITA TTACTOCATA ATAGTATACT NGTACATOTA COTAN COTA CICIONOCO CCATATCAT TATCATATY ACACISTICAGE Terrecorde ACATCAAGTO COTITIACCCO TCCACAGGGT CTTCATGGG TAACACGTCT TGGAGGTCCC CATTICCAAG CTAAAGGTTC CTCCATAGAA TCCCGAAGAC CCTCCAGGSA GCAGGTCCCT AGGCTCTGAG TCCGAGACTC ATTONOONOO GCACCICITIC TAACICCICC ACCITCCAGGG TGTAGTTCAC CCTACTTOGC GGATGAACCG COCCUCOCOCC COCTINAAAGC CULTINCOTO CCANATGCAC TACOTTOTAT ATCCAACATA ATTACGGGGT TAATGCCCCA **GCAMATGGGC** TGACOFFFFF ATTOTOTOGG CCATTICGTG TAACACACCC CCCTCCAAAC GAGGICGGGA GOGACGITING CCTOGAGAAG ATTOTOCAGA GTACCCACGA GCCCAACGAC CCCCGCCAT CATTCTARAC GGACTTTRGAC CCTCANACTO ATCCATTGCA ATAGTAATCA ATGGGACTIT CCCCATTOAC GOOGTAACTG CHGITITICAC CACCOTITIC GTANGATTTG TAGGTAACGT TATCATTAGE COCCOCCTA ACTTOCCAGE TOMOCOSTCA TACCCTGANA GACTCACGGG CTGAGTGCCC THICHCACT MACACTRICA CTCCCNANG GGTTCCTCCG GAACTACCCC ACTETAGATO CCTANAGCAC CTCCAGCCCT CTACTTATTA TAAACTIGCCC TTACCCACCT CATAMATIXC ATTIGACGGG ACATGACCTT TCTACTCCAA TATICOCCANA TTGTTGAGGC CGGTAGGTGC CCANGGAGGG 9000000000 GATCANTAAT CGGGTTGCTG ATAGGGGGTTT AACAACTCCG GCCATCCACG 222222222 CCTANTICCAG CCCCACCTC ACCEPACECAT TOSCHOOLTA CHUTAACCGA ACTICCIATGG TCCCCGCCCC CCCATATION CTATTTACKS GCGGACCGTA ATACGGGTCA ATTENDED GTCATGTAGT TACCCCCACCC GGGACTTTCC ANATISTEGT CCCTGAAACG TTTTACAGCA GCCTGGAGAC COGNICETER CHRICKAARAG CACCATTACTIC GENERALANDA MEANETACAA TUTTICATE CCTGGGCCAG CATACCCCACTC GTGAMAGACA CACTRECTER AGGERETICA TGANTGCCAT TTACCGGGG GACCGACTGG CCANANTCICG CCTACAACAT CATTFAAACC TATTATTATA GTAATGGCGG TACAACTGTA ACTAATAACT CHRIGHTRACE TATGCCCAGT CITANATTING ATATTTGTCT TATAMACAGA GCCCCAGTTT CANCCCCAAA ATAATAATAT TRATTATITA TATTITICAL TGANSCIANT ATGATANTA ATAMAACCTA ACTITICATITA TACTATTACT ACTIVITY EXCUST ANCACATOTA TCACACCGCC TTGTGTACAT GTTTAGTGAN CCGTCAGATC GGCAGTCTAG CHCCCCCCCC GGGNACGGTG CATTGGNACG CGGNTTCCCC GCCTAAGAGG CCACCGTTCT GCAGGCAGAT CCTCCCTCTA CTGTCCACCA GANGATTGAT CTITANCTA AAPTITCACIFIC TINNET ATACTCATA GGATGTTGTA COTTONICATIVE ATCTTCACAT ACTIACGGIA ANTEGCCOGC ANTOCOTOCA CCCCTCCCAT CAGTACATCA ACACAATGAG TATENINGINT CAGGITCITY TICCOGGICG TCCGACGACG ACCGIGICCG CCACTCCCCA AGACTCCCCA TOTICACACIC CACACCTRCCT PUTCHTACTIC CCCAGGTCAAA **OTANCTICIO** CATTTACCIOS GGTACCACTA COCCANAACC GITTINGCAC CANANICANC GTAACCTTGC GCTRAAGGGCT TCTCACCCCT CATTACCGCC CATTRIACGINE CITABANTICACC **CCCCTTTTCC** GTTTTAGTTG CANATICACTT TriATriTIVICA ACTACAACGT CCTITITIAGGG AGAAGATCAG CGACCTCTGG ACCUTATACT AGGENGETES CCATCGTGAT CAAAACCGTG AGCAGAGCTC TCCTCTCGAG CCCTTGCCAC TCTTCTAGTC **GCTOGAGACC** TOGOACATGA TCCCTOAAAO **GTCANTGACG** CAGTTACTGC ANACACICCNA TCATGTCCAA AGTACAGGIT COCMATGTAT ACCORPTITIC GGCGGWATTS TAMARGERIC TCANTANT ACTTATTAM THECOURT GCGTTACATA TAAFATACT ATTATATA CCCCCTTCAC ATTTRICKLY CACAAGTGGG ACCETOGOCT **GOTCTATATA** CAGGGGGCCCGG CHSTITCACCC TOCGACCOGA CACAGTOGCT GTGTCACCGA MOCCCAGC TACCCTICADA CCAGATATAT TICTTANTTA ACATCATCAA CATCATCACA TICACTITIAG ANTATAACCG TACCTCAAGG TAACOCCAAT ATTOCOGITA CCCTATTICAC GOGATAACTO ATCCCTATTA TACCGATAAT ATCCCACTT **OGRAFITGACA AAGTGAAATC** TCTCAGGTGT AGAGTECACA TTATATTGGC ATCCAGTTCC **CCTTTCACTGT** GTAGTAGTGT CCCTGTACAA PFIGCTGTGA **GOGACATGTT** GTCCAAGAAG rectolocito **PATACATIGTA** ATCCCCTATA GTTCCCATAG CAAGTACGCC STICATOCOG CGTATTAGTC ATTOACOTCA **FACOOTOOOA** MOCCACCCT CCGATCCAGC COCTAGGTCG ACCACTOGAC MACGACACT FACCCONTAI CANGGOTATIC **GCATAATCAG FAACTGCAGT** GTCCACAAAA ATATGTACAT CCCCCACTG CCACATGTGT CITAINCHOC CAGGIGITIT ANCANTTAAT COCCUCAC GOTOTACACA GAATAAGAGG 1301 1501 1601 1001 1101 1201 1401 HO1 601 701 901 101 201 401 501 301

Figure ISA

# PMRKAI Sqaq MER682

1701	CACCAGGCCA	Terececes	GACCCTCAAT			ACTITATIVES GRAGAAGGC	Therecette	AGGTGATCCC	CATGITICAL	GCCCTGTCTG
•	6166151661				T.1.W.1.M.1.TL		שאוניאניוער	TECACTAGOS	GIACAMARA	רואאייתראטער
1801	TECCACGGTG	CCCCCAGGAC	GACTERITIES	ACCIAL TITISTIC	MCMTTTTTTC	נידאה ארממידה ב	CCATCCACAT	CGACTTCCTC	ACCATCAATG	AGGAGGCTGPT PCCTTYTGAC
1901	TGAGTGGGAC				CERTAINS		CECCAGGRAG	Tereacarte	CTARCACAC	CICOCOCICI
	ACTCACCCTG	-			נושאטארומנ		CKAGTCCCC	AGACTIGTAAC	GACCCTGGTG	GAGGTGGGAVS
2001	CAGGAGCAGA	-					ACKERTICACITEA	<b>PECTURACET</b>	GANCAAGATT	<b>GTCACACATTC</b> T
	Greenegren		CHEGITICATIO	COMPACTAGE	מאנאניכניכיד	TTAGATGTTC	TCCACCTAGT	AGGACCCGGA	CTIOTICIA	CACTCCTACA
2101	ACTOCCCCAC						TGGACAGGTT	CTACAAGACC	CTCAGGGCTG	AGCAGGCCT
	TOAGGGGGTG	CAGGTAGGAC	CTGTAGTCCC	TCCCCCCCCTT	CCTCCCCAAG	TCCCTGATAC	ACCTICACCAA	GATGTICTOG	GACTCCCGAC	TCOTCOGAG
2201	CCAGGAGGTG					CAGNATGCCA ACCCTGACTG	CANGACCATC	CTGAAGGCCC	TGGGCCCTGC	PECCALICETO
	OGTECTICEAC					CICTIACOCT TOCCACTOAC	CTTCTGGTAG	GACTTCCGGG	ACCCOGGACG	ACGGTGGGAV-
2301	CTCCTCTACT	TGACAGCCTO	CCACCACAC			CHEACAAGC CAGGGTGCTG	GCTGAGGCCA	TOTCCCAGGE	GACCAACTCC	GCCACCATC.
2401	TCATGCAGAG				CAACTOCTTC	GRANGACAGT GANGTOCTTC AACTGTGCA			NACTGTAGGG	CCCCCAMBUAY.
	ACTACGICTIC	CCCGTTGAAG	recriecier	CCTTCTGTCA		CTTCACGAAG TTGACACCGT	TCCACCCAGT	GTAACGGTTC	TTGACATCCC	GOCCOCCCT
2501	GANGGOCTOC	TOGAAGTGTG	GCANGGAGG		CCACCAGATG AAGGACTGCA ATGAGAGGCA	ATCAGAGGCA	GCCCACTIC	CTCCCCNAA	retroceere	CCACAACACA:
	CTTCCCCACG	ACCTICACAC	CGTTCCTRCC	GGTGGTCTAC	TICCIGACGT	Precidades pactenees	CCROTTGAAG	GACCCONTIN	AGACCGGGAG GGTGTTCCCX:	Generaleceer
2601	AGGCCTGGCA	ACTICCICCA	GTCCARGCCT	GAGCCCACAG	CCCCTCCCGA	CCCCTCCCGA GGAGTCCTTC	AGGTTTGGCG	ACCACANGAC	CACCCCCAGE CAGANGCAR	CACINADCAC
	TCCCGACCCT	TGAAGGAGGT	CAGGICCGGA	CTCCCCTCTC	GCCCACCCC	CCTCAGGANG	TCCAMCCCC	recremeno	פונסספפורכם הוכוורכהכי	GICTICGICO.
									-	3,01
2701	AGCCCATTGA			CCTCCCTGAG		CUCANTIACC	CCTCCTCCCA	GTANNATAAA	OCCCOGGCAG AICTOCTOR	ATCTGCTGTK:
	TCGGGTAACT	GTTCCTCGAC	ATCCCCCCACC	CKINGGGACTC	CACCCACAAA	CCGTTCCTGG	CCAGCAGGGT	CATITITATIT	COCOCCCOLC	TAGACGACA
2801	CCITICIAGES	-				GACCCTGGAA	GGTYCCACTC	CCACTGRCCT	TTCCTAATAA	ANTCACCAL
	GGAAGATCAA	COCTCOCTAG	ACANCANACG	CCCACCCCCC	ACCIGNAGGAN	CTGGGACCTT	CCACGGIGAG	GCTGACAGGA	AAGGATTATT	TTACTOCTOR
	,									Sphi
2901	TIGCATCOCA			AGGTOTCATT CTATTCTVAG GGGTGGGGTG GXGCAGGACA	CCCTCCCCTC	CHECHENICA		CCATTICCCAA		GCCATCCTGG
	AACGTAGCGT	AACAGACTCA		TCCACAGTAA GATAAGACCC	CCCACCCCAC	cccencenst	cernececer	CCTAACCCTT	CIGITATEGE	CCCTACGACC
	•		Pari	Asci						
3001	GGATGCGGTG	GOCTCTATEG	CCGATCGGCG	GGATGCGGTG GGCTCTATGG CCGATCGCGTA CCCCGTACTG AAATGTTTS; GAGTACTTA AGGGTAGAA AGAATATATA AGGTGGGGGT CTTATGTAGT	AAATCTCTX;	COUNTRY	AGGGTCGGAA	AGNATATATA	AGGTGGGGGT	CTTATGTAGT
	CCTACGCCAC	CCGAGATACC	GCCTAGCCGC	OCCTAGECTO GEORGATICAC	TTTACACACC	TTTACACACC CXCACCGAAT TYCCACCCTT TCTTATATAT	TUCCACCUT	TCTTATATAT	TCCACCCCCA	GANTACATCA
									Sphi	
3101	THURATUR		0000000000	THITICAGCA GCCCCCCCCC CCATHANGACAC CAAACHTCTITI GATGGAAGCA THGINGACCC ATAITINGACA	CAACTCCFTT	GATGUAAACA	<b>THURSTICAC</b>	ATATTTCACA	ACGRECIATES CONCATARES	CCCCATAGGC
	AAACATAGAC		2002000000	AAAACCTCGT CCCCGCCCCC GCTACTCCTC GTTGACTAA CTACCTTCGT AACACTCGAG TATAAACTGT	CITTGAGCAAA	CTACCTTCGT	ANCACTEGAG	TATABACTOT	TECCCOTACO GOOCTACCO	<b>GOOGLANCE COM</b>
3201	COCCUTCCGT		TOTAL TOTAL	CAGANTOTGA TESSATTITICAG CATTGATEST CHITCHITT THECTSTANA CTCTACTAGE TIGACCTACG AGACCOTOTC TIGGAACGCG	حديدددنيسد	TERCOLACAAA	CTCTACTACC	TTGACCTACG	AGACCGTCTC	TOCAACGCCG
	CCCCACGCA	GTCTTACACT	ACCCGAGGTC	BOCCCACGCA GTCTTACACT ACCCCAGGTC GTAACTACCA GCCCAGGCA ACGCGCGTTT GAGATGATGG	CCCCCCCCCACACAC	ACCOCCUL	GAGATGATGG	AACTOGATGC	TCTGGCACAG ACCTTGCGGC	ACCTAGGGG

tique 150

	Pstl	118		Psil						
TTOO T	PLOGAGACTO	CARCCTCCCC	CCCCCCTTCA CCCCCCCAACT C	מאנאראות בראריולות מארארארים בראריולות		ר האהאארויה א ה האאליויה א	ACHERCETATION OF	GNAATSIACTE G	GECKALTINGCA V	THYTIC ACCITIC
	CHICCOCHIC		CATCACAGE	TCACCCCTCT 7	TITIVA:CAL'AA	ANCCTANGAL I	TICACICICATION PACTICICATION PACTICICATION PACTICATION	ACTTANTOTO O	CAANGAGTEG .	ACKTRITICATA TEGACAARI "F
	retococcao Agangangan		CCCTCAAGGC		-			CCAGACTCTG 1	TTKGATTTG AAACCTAAAC	GATCAAGCAA CTAGTTCGI I'
CAC	GIGTCTTGCT		AGGGGTTTTTG TCCCCAAAAC			הכאסמדידרד ( מקדניגנינאסא (		CCCAGGACAC A	TATITITICC ATABABABABABABABABABABABABABABABABABABA	AGGACCTA:T TCCTGCACCA
3 }	AAAGGTGACT TPTPCCACTGA	CHOGATOTIC	AGATACATGG	CCATATICOSO	CAGACACA C	TECHNICITAGE	ACCACTRICAG ACCTTCATCC TCCTCACCTC TCCAAGTACG		TCCCGGGTGG ACGCCCCACC	tgttgtagat Acaacateta
3 t	GATCCAGTCG		GCTOCHGCGTG	CACGGATTET	ATCICITION	GTACCAAGCT	GATTIGCCAGG CTANCGGTCC	GCAGGCCCT 1 CCGTCCGGGA 1	TCOTOTANOF	GTTTACAAN: Caaatgttac
5 8 8	COCTATICGA		CATACGTCKSS		GCANCTINGA CGT/NJAACCT	CHUTATTITI	ACCITICOCTA TECANECGAT	TOTTCCCAGE O		CCCCTAN:T
<b>F</b> =	TOTTOTOCAG			CCCACCTICAC	GGGAAATTTG	TCATGTAGCT AGTACATCGA	TAGAAGGAAA	TOCCITOGRAG I	AACTTGGAGA TTGAACCTCT	CCCCTTGTVI
<b>2</b> F	ACCTCCAAGA			AATTAATGGGA TTACTACGGT	ATTAPACTICAC TACCOGGTG	CCCACCACCG	CHASCCGANG	ATATHTCTGG (TATANAGACC	CHACTCATTO	GTCATACTTG CAGTATCAA("
<b>F</b> 2	TOTTCCAGG			TTTACAAAGC	COCCCCCCCCAC	CCACGGTCTG	TCCCCTATAA	TOGITICCATC ACCARGO (	COCCOCCICCC	GCCTACTTA CCCATCAA'N:
	CCTCACAGAT		_	GTTCAGATGG	CCCCTAGTAC	TCTACCTGCG AGATGGACGC	GGGCGATGAA	GAAAACOGIT	TCCOGOGTAG AGGCCCCATC	OCCITCTAGTC PSI
00.	CTGGGAAGAA GACCCTTCTT	A AGCAGOTTCC T TCGTCCAAGG	TCACCACCTC	CCACTTACCG GCTGAATGGC	CACCTOGITGG	CACCTOSTICE GCCCGTAANT CACACCTATT CPCCCCCACC CGGCATTTA GTGTGGGTAA	CACACCTATT	ACCECCACGE	ACTOSTACTE TGACCATCAA	AAGAGAGCA: TTCTCTCGAC
- ; 0 6	CAGCITACOGO GICCAACOGOA	T CATCCCTGAG A GTAGOGACTC	CAGGRAGGCC	ACTICGITIAN	GCATGTCCCT	ACTICGITA GCATGICCT GACTCTANG IGAAGGAAIT CGIACAGAA CIGAGGGIAC	TTTCCCTGA AAAAAGGACT Spib	CCAAATCCGC	CAGAAGGCGC	TCGCCGCGCT AGCGGCGGGT
60	OCCUPATION CONC	G TICTTOCARG	GANGCAAAGT	TTTTCANCIO	TTTGAGACCG AAACTCTTAGC	POCCCCCTAG NFXXFACATC	GCATCGANAA CGTACGANAA	GACCUTITICA CTCCCAAACT	CCAAGCAGTT	CCAGGCGGTC GCTCCGCCA!!
00	CCACAGCTCG	_	T CTACGGCATC	TCCATCCATC ACCTACATICG	ATATCTCCTC TATAGAGSTAG				CCCCAGTAGT	CCACGAGGA
טט	CCAGACGGGC	C CAGGGTCATG	G TETTTECACE	CCCCCTCCCA	CCTCTTTTAGG GGAGGATTG	CATCAGACIC	TCACCCTCAA	CCCCACGCGA	CCCCCCACCC	CCC TRICK CW.

figure 15c

<b>CONGCOCTIV</b>	0					ניכונטלוטכניוכ	CCCCACATAG	CATTRICACCA	TCCTCTCATA	CACCAGGGGG
	1 1				ACTION ACTOR	נאיישרנייריוריים				GCGAGARATA
z. F.	TCCGCGCGT GGC AGGCGCCGCA CCG	CCCCCTTAXC (	CCCOTCGAC	CCCTTTTTCACT	אושיניין וניל.א דרכימרמינינד	CX TRICRICATE	ACCTUTANA			CCCTCTTTAT
	to		TCCACGCCGC	AGGCCCCCCCA	GALTATION	CATTCCACGA				ANACCAGGIT
	ย		AGGCGCGGCG	recedencer	CTRCCAGAGE	GTVAGGTGCT	COCICCACTE	GAGACCGGCA	AGCCCCAGTT TT	TTTGGTCCA!
	TCCCCATGC TIT	THITGATCC	GPPICTTACC	TCTCCCTTTCC	ATHAMACCINGT	GICCACGCTC				TACAC: ACTTY:
	13		CANGANTOG	ACACCAAAGG	TACTICACCA	CARGITECGAG	CCACTGCTTT	TCCGACAGGC	ACAGGGGCAT	ATGTCTGAAC:
	* }	Xyot Market								
	AGAGGCCTGT CCT	CCTCGAGCGG	TOTTICCIACGG	TECTECTOR	ATAGAMACTC	GUACCACTCT	CACACAAAGG	CICCCCITCCA	OCCCAGCACO	AAGGAGGCTA
	TETECCOURCA GGA	<b>GGAGCTCGCC</b>	ACANGGEORCE	ACCAGGAGCA	TATCTTTGAG	CCTYGOTGAGA	CICIOLLICC	CAGCCCCAGCT	ccconcence	TICCICCOAT
	O	GTAGCGGTCG	TTGTCCACTA	GOGGETECAC	TUTCHCCAGG	MCTGAAGAC	ACATGTCGCC	CTCTTCGGCA	TCMGGMGG	TOATTOSTIT
		CATCGCCAGC	AACAGGTGAT	CCCCCAGGTG	MACHANISTICC	CACACTTCTG	TOTACAGOOG	GAGAAGCCGT	AGITICCTTICC	ACTAACCAMA
•		OCCACOTGAC	COGGRETICC	TGAAGGCGGG	CTATAAAACG	GOCTGGGGGC	acenteence	TCACTCTCTT	CCGCATCGCT	GICTOCCAGG
3	Ų	COGTOCACTO	GCCCACAAGG	ACTIVCCCCC	GATATITICS	CCCACCCCCG	CCCAACCACC	ACTCACACAA	OGCOTAGCGA	CAGACGCTCC
7,	1	GGGGTGAGTA	CTCCCTCTGA	ANAGCOGGCA	TENCTICING	GCTAARGATTG	TCAGTTTCCA	AAAACGAGGA	CONTITIONTA	HCACCAGO"
	5	CCCCACTCAT	CARRENGALT	THYCGCCCGT	ACTGAAGACG	CCATTCTAAC	ACTCAAAGGT	THINGICA	CCTAMACTAT	AACTGGGACCYI
							Hingel			
	CCISCOGROAT OCC	OCCUPAGA00	PROCECCAT	CCATCTORTC	AGNANAGACA	ATCTTTTING	TOTCANCETT	TOTCANCTT GGTGGCAAC	GACCCCTAGA	GERCUTTION
	2	COGNAACTCC	CACCOGCGTA	CCTAGACCAG	retrinence	TAGAMMACA	ACAGTTCGNA	CCACCGTTTIG	CTGGGCATCT	CCCCCAACCT
					Pwil					
	CACCACTTO OCO	OCCATOGAGE	<b>CCACKOTITIC</b>	GTPTTTCTCG	CGATCGGCGC	GCTCCTTGGC	COCCATICATA	ACCTOCACOT	ACCTUCACUT ATTROCCCCCC	AACCCACCK.
	Ų	COCTACCTCG	CGTCCCAAAC	CANADACAGO	CCTAGCCGCG	CCACCAACCC	GCGCTACAAA	TCGACOTGCA	TAAGCCCGCG	TICCCTCCC)
	5	AGACGOTGOT	GCGCTCGTCG	GCCACCACT	CCACCCCCCA	ACCIDENCIA	TOCAGGGTGA	CANGOTCAAC	OCTOGTGGCT	ACCTETECT
	£	TETGCCACCA	CCCCAGCAGC	CCGTGGTCCA	Catacacast	TOXCOCCANC	ACCITCCCACT	GITICCAGITIG	CGACCACCGA	TOCACACC
	GTAGGCGCTC GTT	GITGGTCCAG	CAGAGGCGGC	COCCCTTOCC	CGAGCAGAAT	GGCGGTAGGG	GGTCTAGCTG	CONCINCATOR	GOCOCOTOTO	CCTCCACGGT
	9	CAACCAGGTC	OTCTCCCCCG	BCGGGMCTC	CCTCCTCTTA	CCCCCATCCC	CCAGATCGAC	GCAGAGCAGG	CCCCCCAGAC	GCAGGTTGCCA
	AAAGACCCCG GCC	GGCAGCAGGC	OCCCGTCGAA	GTACTCTATC	THECATERET	GCAACITCTAG	CCCCTCCTGC	CATCCGCGGG	COCCANGCIC	CCCCTCGTAT
		CCGTCGTCCG	COCOCABOTT	CATCAGATAG	AACGTAGGAA	CCTTCAGATC	GCTACACGACG	GTACGCGCCC	accometes	COCCAOCATA
		GOOGACCCCA	TOCCATGREE	TOSCHEAGEG	CCATACATACGTA	CATCCCCCAA	ATCTCCTAAA	CCTAGAGGGG	CTCTCTGAGT	ATTCCAAGAT
		CCCCTGGGGT	ACCGTACCCC	ACCENCINGE	GCCTCCCCCAT	GTACGGCGTT	TACAGCATTT	GCATCTCCCC	CACACACTCA	TAAGGTTCTA
0	٤	GCATCTTCCA	CCCCCGCATATCC	TOCCOCOCAC	GTAATCGTAT	AGENCATACG	ACCCCAG	GACCTCCCCA	CCGAGGTTGC	TACGGGCGC
U	FACATOCCAT COT	CCTAGAAGGT	OGCGCCTACG	ACCOCOCOTG	CATTAGGATA	TCAAGCACGC		-	OCCITCCAACG	ATCCCCCC
$\boldsymbol{\varrho}$	CRECICIACT COC	COGRAGACTA	rerecerson	GATCCCATCT	GACTIVICATO				TRACCIPCTOT	CAGACC-TACC:
Q	CACCAGACGA GCC	GCCTTCTGAT	AGACGGACTT	CTACCCTACA	CTCAACCTAC	TATACCAACC	TOCCACCTTC	TOCAACTTCG	ACCOCAGACA	CTCTGGATO:

Figure 150

# PMRKAdSgag MER682

							The second second		The British of the Party of the	Try To The Affects
6501	COCAGTACOT	CCAMICANOC	CATCCTCAGC (	CHALMINE FINAL CATESTON A	ACTOSTOCIACIONO				CAGGTCCCAA	AGGAACTACT
6601	TGTCATACTT ACAGTATGAA	TAGGACAGG	ANAMAAAGG	ACAGCTCKOO (TENCISAGEGE)	CAACTCCTVAT	AACTETTETE OF THESE OF THE STATE OF THE STAT	CCAGNANGGT	GTACTCT-POG CATGAGAACC	ATCCCAAACC TAGCCTTTGG	CCINCCCCT . OCACCCGA:
6701	CSAACOGTAA	GAGCCTAGCA	TGTAGAACTG ACATCTTGAC	GTTGACGGCC	PRICTAGGGGG	AVECATOCOTT TO TEXT	THETACAGET	AGCCCCTATO TCGCCCATAC	CCTOCOCOCOC	CTTCCGGAGG GAAGGCCTV1:
6801	GAGGTGTGGG	TGAGCGCAAA	CCACAGGGAC	ACCATICACTT TY	TGACKITACTES	CATANACTIC	TCAGTGTCGT AGTCACAGCA	COCATCOGCC	CTCCTCCAG	AGCAAAAAGT TCGTTTTTCA
6901	CCONCCCCTT	MARCTIGGG	GCATTTGGCA CCTAAACCGT	CCCGCTTCCA	CHETAGENAC	AACACATACT	TTY COCCIOCO ANABACICOCOC	AGGCATAAAG TCCCTATTTC	TTGCGTGTGA AACGCACACT	TGCGGAAGGT. ACGCCTTCCT
7001	TCCCGGCACC		TOTTAATTAC	CTGGGGGGGG	ACCAMBATET TOGSTOTAGA	CCTCAAACCC	CANCTACAAC	TOCCCCACAA	TETAANGITC ACATTICANG	CAAGAAGCGT GFTCTTYCACT:
7101	CCCTACGGGA	F TOATOGAAGG	CAATITITIA	AGTTCCTCGT TCAAGGAGCA	AGGTGAGGTC	TTCACOCCAG ANGTECCECTC	CTCACCCCT	CCTCTCAAAG CCAGACTTTC	GGCCCAGTCT	GCAAGATHAG COPTCTACTC
7201	GOTTOGAAGC	C CACCAATGAG 3 CTGCTTACTC	CTCCACAGGT	CACTACACCAT	TAGGATTTGC ATCGTANAGG	ACCTCCTCCC TCCACCAGCG	GANASCHECT	MACTOGOGA	CCTATCOCCA	TTTTTTCTGG AMAMAGACC
7301	CCACTACGTC	G THCMOGTAN	OCCORTETTO CGCCCAGAAC	TTCCCAGCGG AAGGGTCGCC	TCCCATCCAA AGGGTAGGTT	CCANACCCC	TAGGINCINGC	GCGGCAGTCA	CTAGAGGCTC	Atctccccc TAGAGGCGGC
7401	AACTTCATGA	A CCAGCATGAA T GOTCOTACTT Pad	GCCCACGAGC CCCGTGCTCG	TCCTTCCCAA ACGAAOGGTT	AGGCCCCCAT TCCGGGGGTA	CCAMPTATAG GGTTCATATC	GACACTACAT	CCATCCACTG	AAAGAGACGC TTTCTCTGCG	TCGGTGCGAG AGCCACGCT
7501	CTACGCTCO	\$	AACTGGATCT TTGACCTAGA	CCCGCCACCA	ATTCCACTC	TOCCTATTGA ACCGATAACT	TETEGREAAA	OTAGAAGTCC CATCTTCAGG	CTGCGACOCO	CCGAACACTC
7601	CACGACCGAA	T TTOTAAAAC	GROCCCAGTA	CTRGCAGAGG GACCGTCGCC	TCCACCACACT ACCTCCCCUA	GTACATCCTG	CACGAGGITG	ACCTGACGAC CGCGCACANG TOGACTGCTG GCGGGTGTTC Khol	CGCGCACAAG GCGCGTGTTC of	<b>GAAGCAGAGT</b> CTTCGTCTCA
1011	CCTTAACT	A OCCCCTCGCC T COCOGAGGGG	ACCECECAAA	CCCACCACCA	CTTCTACTTC	GCCTGCTTGT CCCACGAACA	CCTTGACCGT CTTAACTKINGA	CTCACTECTC GAGGGAGTT	GAGGGGAGTT	ACCOMICAN:
7801	OGACCACCAC CCTOGTOGTO			AGATOTOCGC TCTACAGGCG Pell	GUIZCUACCUT CHAYAXXIACCA	CCSACCTTON GCCTCGAACT	TCACAACATC ACTGTTGTAG	GCGCAGATGG CGCGTCTACC	GAGCTGTCCA	TOGTCTCOAG ACCAGACCTC
1901	CHCCCCCGCGC	c orcadorcado o caorceagre	OCCOCTCGAG CGCCCTCGAG	CTCCACCTT	ACCTUGG ATA TOCACCCTAT	GACKRICTC	GCCCCGCCCA	AGATECAGGT TETAGGTECA	GATACCT/AT CTATGGATTA	TTCCAMGGGC AAGGTCCCCG
8001	TRETTUSTUS		COGCOTCGAT GACTTACANG AGGICACATC CLOCACAGGIC GACTACACATA CARAGGAGIC COCCOGAGGICATO TOCATACAATA; OCCOCAGCTA CCGAACGTIC TOCCGGGGAAG (ACACACACAGGIC CTGATACGAT GAGGICAGGIC COGCCACCGG GAGGICATAC AGGIAATACAATA	AGGCCGCATC TCCGGCGTAG	בבנאבמטנטנ מסמנטכנענט	MOSICIACATO CONTACARIOS GALTANIANA CA VARIORADO GOOGOGOGO COCOGUISTO TOCITIASANTI. TOCOGOGOTAS GALACICAS CIGANIACIAN CASCAGOGOS COGOCAGOGO GOOGOGOGA AGAMACITAI.	שייי היייי היייי היייי	CCCCACCC	COCCOCCCCAC	TCCTTREATY: AGGNACCTAT

1018	ATCCATCTAA	MCCCCTCAC	GCCCCCCAAC	ננננגאאאנ	ACCEPTANT	בבישני אכב כיים	COCCAGACACS (	GCCAGGGGCA (	בפורפשימכר ו	GCGCGCGGAAC
	TACGTAGATT		כפכנינוצינוני	GREGORYCTCCA	<b>אכיבוג גיוניו</b>	GINCTANGREG (		ccoreceer (	GCAGCCGGGG	دەدەئىددىد
8201	ASSAGENTOR TECHEGACEA	CONCOCCCCC	PARCHIOCHE	COCTACACOTTA	(TIMETATANT) GETEATICINE	CAACTAGAGG /	TCANTETYTE O	OCCTOTOCOT COGNICACION	GAAGACGACG CTTCTGCTGC	המככננגשייוניא ברסמסכנאריוי
1018	GCTTGAACCT	GAAGAGAGT			CANTITICAL CIRCATTIGACE	מינייניינייניינייני	מראאאזרדכ	CTGCACGTCT	CCTCAGTTGT	CTTKWTAGG"
	CGAACTTOGA	CTTTCTCTCA			כאבאיאאכיזיטכ	כטכנטפעככם (	CCTTTTAGAG	GACGTGCAGA	GCACTCAACA	GAACTATCC:
				E	111					
8401	GATCTCGGCC	ATGAACTOCT	CONTETETE		CHUCHNIAGA TCTCCCCCTC	ניטאריווינאכיוכ	CACOGINGCG	GCGAGGTCGT '		GCCCATGAC "
	CTAGAGCCGG	TACTTGACGA	GCTAGAGAAG	GARGACCTCT	AGALKUCGCAG	CCCCAGCCAG	CTICCACCCC	CGCTCCAGCA	ACCTITACGC	CCCFTFACTU:
8501	TOCCAGAGG	CONTRAGREC	Tecenceric	CACACGCCACC	TYTTAGACCAC	פניככבבידוכה	GCATCGCGTG	COCCATOAC		AGATTCACC "
	ACCEPTEC	GCAACTCCGG	AGGGAGCAAG	פתכתכפכבני	ACAINCTIGGTG	CLYCOCKCAAPC	CCTAGCCCCC	CCCCCCTACTG	OTGGACGCGC	TCTAACTCO.
8601	CCACGTGCCG	GOCGANGACO	GCGTAGTTTC	GCAGGCGCTG	AAAGAGGTAG	TYCACACHUS	recenence	TTCTGCCACG	AAGAAGTACA	TANCCCAGO:
	GOTOCACOCC	CCCCTTCTGC	CGCATCAAAG	COTCCOCGAC	TITCTCCATC	AACTCCCACC	ACCCCCACAC	AAGACOGTOC	TTCTTCATGT	ATTIGGGTCCK:
		ŭ	EcoRV							
8701	TCCCMCGTG		GATTCOTTGA TATCCCCCAA	OCCUTCANGG	CGCTCCATGG	CCITCTAGAA				COCCCCC
1	AGCGTTGCAC		ATAGGGGGTT	CCCCAGITCC	GCGAGGTACC	GGAGCATCTT	CAGGTGCCGC	TICAACTETT	TGACCCTCAA	COCCCCCCTC
8801	ACGGTTAACT	r cereereed	MGACGGATG	AGCTCGGCGA	CAGTATCOCG	CACCTOONIC		CAGGGGGCCTC	TICTICTICT	reastere:
	TOCCAATICA	CCAGCAGGTC				GTKGAGCGCG	AGTITICCGAT	GTCCCCCGAG	AAGAAGAAGA	ACTTAGAGGA
									Spi	2
8901	CFFCCATAAG	AGCCTCCCCT	Tefrencia	בומכנסבכם	THACARCACACAC	GCGACACGC	GCCGACGACG	GCCCACCGCC		CAMACCETE
	GAAGGTATTC	CCCCACCCCA	AGANGANGAN	מערפככפכ	ACCCCCTOCC	CCCTGTGCCG	CCGCICCTGC	CCCCTOCCCC	TCCGCCAGCT	GFFFCCATGAG
1000	GATCATCTC	C CCGCGGCGAC	CCCCATGG	CICCOTTOACC	פרהההההככה	TUTCACCCCC	GCCCAGTTCG	AAGACGCCGC	CCGTCATGTC	CCCASTTATOS
1	CTAGTAGAGG			-		AGAGCOCCCC	COCCUTCAACC	TTCTGCGGCG GCCAGTACAG	CCCAGTACAG	GCCCAATACC
9101	GTTOCCOCOC	G GCCTCCCATG	CUCCAGGGAT	ACCCCCTAA	CGATTECATET	CAACAATTGE	Transtrancera		GAGGGACCTO	AGCGAGTCT
	CAACCGCCCC	C CCCACOGTAC	accencera	TOCCGCGATT	GCTACGTAGA	GTTGTTAACA	ACACATCCAT	GAGGCCGCCGG	CICCCIOGAC	TCGCTCAGG
			XHOL							
9201	CATCUACCO	G ATCGGAAAC	·t	ACCCUTCTAA	CCAGTCACAG	TOCCARRETA				GCCGGTCGG#:
	GTAGCTGGCC	TAGCCTITIO	GAGAGCTCTT	TCCGCAGATT	GGTCAGTGTC	ACCUTICCAT	CCCACTCGTG	GCACCGCCCG	CCCICCCCC	CCGCCAGCCC
							Saff			
9301	GENOTIFICIO	3 GCGCAGGTGC	TOCTGATGAT	GINATTANG	TACKACKATACT	TYSAGACTGCG	GATGGTL: FLAC	AGAAGCACCA	TOTAL	TCCGGCCTGC
	CAACAAAGAC			CATTAATTE	ATCUTACAGA	ACTOTOCOCO	CTACCAGCTG	retregion	ACAGGAACCC	AGGCCGGACG
9401	TCAATGCGCA	A OCCOURGE	CATCCCCAG	GUTTCOTT	CACATOCOCO	CACATACTERS	TACTACTO	GCATGAGCCT	TICTACCOCC	ACTIFICATION
	ACTITACGCGF			CGNAGCANAA	CTGTAGCCGC	GTCCAGAMAC	ATCATCAGAA	CCTACTCGGA	AAGATGGCCG	TGANGNAGA
9501	CICCIIICCII	C TIGICCIOCA	TCTCTTGCAT	CTATCGCTGC	נאנגענטטטנט	CAGTITICATE	CTARCTIGGCG	CCCTCTTCCT	CCCATGCGTG	TGACCCCGAA
				ו מאדאנאנקאכם	בניואכניונכנינונ	CICANACIARS	CATCCACCC	CCCAGNAGGA	CCCTACGCAC	ACTGGGGCT I
1096	GCCCCTCATC	C GCCTGAAGCA	מאכדאפפדכ	: משנמעכעעכם	COCINGOCIA	ATATORCOTO	CTRICACCTGC	GTGAGGGTAG	ACTURONGIC	APCCAPATECY
	COCOCACTAC	3 CCGACTTCGT	CCCGATCCAG	CCCCTGTTGC	GCGAGCCGAT	TATACCOGAC	GACGTGGACG	CACTCCCATC	TCACCTTCAG	TACKTACACO

Figure 15F

CYNY GETANDOGC CONTINGATO GIVINAATIIC AGTICACCAT AACAGAAG TIAACAGICT GOIGAGCGG CIGCGAGAGC ICGGIAAFAC	EGCCA CCATACOCOO GCACAACTAC CALATTICACO TCAACCOUTA THICCTOSTIC AATTGCCAGA CCACTORGCC GACACTCTICG AGCCACATOR
GOTTOACCCGG	CCACTORGCC
3 TTAACGGTCT	C AATTGCCAGA
T AACTERACTA	א זידאירי א
. אניבנוניניניניא	TCAACCUIT
CHETANTICE	CACATHUMO
CGREETGATG	GCACAACTAC
GETATISTICS	CCATACOCOG
ACKARACTOR!	TOTTTCGCCA
1070	

	SCOTTAGAC CGCCATCTC	
A CCATACCCC CCACATTLAKE WARLENING THE CONTROLL OF THE CONTROL	What CHANDAGE CHANDAGE CHANDAGE CHANDAGE GEORGE CONTROL CHANDAGE COCCOCCO CONTROL CHANDAGE COCCOCCOCCO COCCAPETE CATEGORY COTTOL CHANDAGE COCCOCCOCCO COCCAPETE CATEGORY COTTOGORY COTTOGORY COCCAPETE CATEGORY COTTOGORY COTTOGORY COTTOGORY COCCAPETE CATEGORY COTTOGORY COTTOGORY COCCAPETE CONTROL CATEGORY COTTOGORY COCCAPETE CO	
	CANAAAOTGC 3 GTTTTTCACO	
	FACT GGTATCCCAC ATGA CCATAGOGTA FGATA	
	C ACCARATAN; or TRAFFICATE or the features.	
מ איאר כניייין	T GTAAGACCG A CGTTCAGGG	
C CACATITIONS	A CCTACTICATY T GCATCACTA	
S CCACACATA	Wel CTC GAGTCAAAT GAG CTCAGTTTA	
CCATACCCC	GTAAGCCCTC CATTCGGGN	
TOTTTCGCCA	GAGACGC	
	9801 T	

1	P. 1	ર		ë.		
			ושכנינינפני	ددودورو		
	COTCAT C	CCACTIA	ככנופנוב ז	GCCAG 1		
	TCCA	150%	5000	CACC		
	TACC-TOGAC!	ATCCACCTG	TCGGGACGC	ACCCTTCCG		
	נינידאניאדט	SCATCTAC 1	CTCCATEG !	CACCTACC		
	T AGGINGOCO GOOTTCCCARE GALGACANTET TECANENTAN GALGATEATA TECHTARATE TACCTOGACA TECANOTICATE OCCORRECTOR	A TECHNOCOC CECCAGGECE CEGETETARA AGGINGTOTAT CEGETACTAT AGGINETAS ATGGACTAT AGGINETAL CANCENTAL	R CHINING GENERAL GENERAL GENERAL CONTINUE TO CONTINUE TO	SCHOOL ACTION OF THE PROPERTY	, , , , , , , , , , , , , , , , , , , ,	
	<b>K</b> 134	t cc	אָט כיאַ	ָּבָּ בַּב	֝֝֝֝֝֝֝֝֝֓֓֓֓֝	
	TCC AAC AT	AGGTTGTA	וניווניניניני		M. Michel	
Management	XXXCACATCT	COCTCTAGA	CKETTECAGA		SC. AMOUNT	
	SCTCCORG C	CCAGGCCC C	COCCEPTOR OF		מכפרבונים נ	
	200	200	DAAA GI		È	
	COCOTOC	CCCACC	13 13 13		20000	Xbs
	COCCAOCGE	CCOSTCOCA	) Self-salatage		CCACCTCC	
	2000			3	505	
	1000			10001		

	ANTICOMOS ANTICOMOS ACACIONES ACIONACIONES ACIONACIONES ANTICOMOS CONTINUES CONCONCONO	A CHICAGO CONTROL CONT		THE THE THE PROPERTY CHICAMPTERS FEGIVALITY CHICKFITTY BAY CEARCING TREGARDED GACAACOOKS GAGINETT	CONCONCIO
Contract Street & Street	Services	CCATAGTACC		CACAMCCOCC	ACCOUNTS CONTRACT TO CONTRACT OF CONTRACT
-	AATTECCAMO	TTAAGCGTTC		TOCCACCICA	P CONTRACTOR P CONTRACTOR
	CHESTINGATA	CALTACTAR		ANTICAGATIC	010000000000000000000000000000000000000
	CTTCCGTCGT	CRACTARTA	1	CCCCCCTCTC	
	ACCOUNT ACT	Bergerer Property B.	זרטרניניוייי	CCCTTACTOC	
	ACACCCTCTA		TC TCKKGACAT	CHAC PARTY WING	
	CHESTAAAAGG		CACGITITICC		2000
-	Campanage	Service and the service and th	CCACATCTOD		CLCCIAICLG
	A deventeday!		TTAGCAACTG		CONTINUENCE
	****	TOTOT		,	10201

THE CONTINUES OF STREET ACTIVITY OF THE CONTRACT CHARACTER AND ANTERCOME THAN STREET CONTRACT CONTRACT CONTRACT CONTRACT TO CONTRACT TO CONTRACT THAN STREET CONTRACT CONTRACT CONTRACT CONTRACTOR CONTRACT TO CON	CONTINUED COCUTATORS GOOGLOCOTIC GIGALICADE COLITACIOC COCOCITATA ANTOCAGA GACAGOSTA GACAAGOGG GAGACOTICATA COCAGAGA GACAGOGGA GAGAGAGA GACAGAGA GACAGAGA ACOCAGAGATA COCAGAGAGA COCAGAGAGAGA COCAGAGAGAGA COCAGAGAGAGA COCAGAGAGAGA COCAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA	THUGGINGE THECAGGEGE GGEGGENGET GEGENACTY THITTHECEAE TRYCCOCEG CALCONAGE GGINGGEG GAARGEGAAA GEATHANGTH.  ANACEGNAG ANGTECEGE CECECUAGA CECEATEGA ANALOGING ACCEGEGE GICGEATTEG CEATEGAC CHINGETH CETAINTER.
NTA ANTICCC	THE TREGACE : AC ACCETUCA	NGC GGTTAGG
ST CTCATING	THE ANTECAGE	CC CACCCATA
T CTTCCGTGCACT	C CCGCCTITTE	C TRACCOCO
AGCCAPATANT TCGCCCCFICA	COCTTACCO	NAMCCOST
AGAGGCTGTA TCTCGGACAT	GTCATCCATG	GCGCTAGCTT
GTGCAAAAGG	GCCGFCCACC CGCCAGGCGG	COCCCCACCA
CTCTAGACC	CCCGTATCCG	TTCCAGGGGC AAGGTCCGCG
AATCGTTGAC C	CCCAAGCTCG	PHEGETTEE ANACEGANGO
10101	10201	10001

H	TAGCAACTG	בפאמאורושת הארפוווורר זרורישטראו והתרכניייי	Cataline Cataline	ורוניסארטי			berre Berrie	LINESCONER C	CACAACGGGG	GAGTGCTCCT
600	<b>GCGTTTCGAGC</b>	CCCCTATCCG	<b>GCCG1</b> CCTCC	GTCATCCATG	COGITTACCE	CCCCTATCC GCCGTCCTC GTGATCCATG CGTTTALCTA CCCCTATION WAS CLASTIC TO COLOR OF THE CO	VK CLAPATIO	o concord		CENTRACTOR
Ş	CCAAGCTCG	GOCCATAGGC	COCCYGGCGG	CACTAGGTAC	GCCNATCACKG	GOCCATAGOC COCCAGOCOS CACTAGATAC OCCANTANO GACKOAGOC TRANSFICANO ACCITAMAL LIGITACOC CONTRANSFICANO ACCITAMAL CIGITACOCOC	PRAKTICCAC A	ארכירופראפו	רופוומררר	
E	PHYSICHICS		OCCCCTOCT	GCGCTAGCTT	PTTTCACCAC	THE MANGE BEGGETICH GEGETACHT TITHERECAE HARCEGEGIG CMANCHANGE GOTHAGATH GAMAGEBAN GENTAURING	CARCCTANGE	GETTAGGETG	GANAGEGANA	GCATT/VICIT:
Ž	ANACCGANGO		CCCCCACCA	CCCGATCCAA	MANACOSTIC	ANGITECEGO ECOCCONCOA CECONTEGAA ANANCEGING ACCOCCOGO GEOCCATTEG COANTECOAC CTINGETH LEIMAILLAN	STCCCATTCG (	CCAATCCGAC	CFFFCGCFFF	CGIMATICAL
E	GENERALINGE	TCTACCCGGA	COSTITATIFF	CCAAGGGTTG	ACTICICIONA	TOTACCEGIA CASTIATITY CCAAGGETTG AFFICEARIA CCCCCGGTTC CAGTCTCGA CCGCCGGAC TACGCGAMC GGGGATTGT	אמוכונכוני	CCCCCCCCCC	TOCOGCGNAC	GGGGGTTIG
8	COAGCGAGGG	ACATOGGCCT	CCCANTAAAA	<b>GCTTCCCAAC</b>	TEMACGCCCT	ACAMOGOCO CCCANTADAD GGTTCCCADO TENZOCCOT GGRAGICANO CTCAGAGOCOT GGCGGGCTIG ACGCCATTIV	CTCAGAGCCT	מככנפפכבום	ACCCCCCTTO	בררר אמניי
•	Carried Carried	TENTARGACIC	CCCTTCCAAA	TECTECHEA	ANCAGGGAGG	WEVARENCE CETTIENAN TECHCOREA ANGMOGACE ACCECTETE TECETITEC CAGATECATE COSTICIOS GCAGATACO	PRCPPPPCC (	CAGATTACATTC	CGGTGCTGCG	GCAGATACO
֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓	Cicconst		CACCANCETT	AAGGAGGCCT	THETCCTIGG	ANTHINESS SYSTACTITY ARGARISCE THYTICCTIC TCORGGANA ANCGANAGE STCTACTING GCCACGACG	AACGAAAAGG (	OTCTACGTAG (	CCCACCACCC	COLCTACOC
5			ACAGT BACAG	CASTERANGA	CATCACAGGAGG	******** PRATEBURG FARTHERING CATCHOCCT COTCOTACC COTCAGAGO GOOGRANCE GOOGTHORES	CCTCCTACCG	CGTCAGGAGG	GCCGACATCC	GCGGTTGACG
3 8	כניניניניני	MOCMOCOCCO.	TO THE COLUMN	GICACCOTOT	GTACCTCCCG	PROPERTY THATTHEE GEOGRAPH GRANGING TREGORDERS GRANDING GEOGRACIEC COOLUMNS COCCANENT	GGAGGATTGC (	GCAGTCCTCC	CCCCTGTAGG	CCCCMCTYX
3 8		TESTUALIA	GAACCCCCCC	202222000	CCGCCACTAC	HASHARTAC GARCCCCCC GGCCCGGGC CCGCCACTAC CTGCACTTCC AGAGGGCGA GGGCCTGGG CGGCTAGGAG CGCCCTCTCT	ACCIAGGGCGA	0000000	COCCTACIONS	COCCURTO
i e	SCOREGICT.		CTTGGGGGCG	CCACGGCCCG	GOCCOTGATG	ACCACIPATE CITICAGONG CONCOCOS GOCOTIONES (ACCITIANZO TECINOCOCT COUGAINCOC GOCOTIOCITO GUILLAGAM)	TCTCCCGCT	CCCCAGNCCCC	OCCONTCCTC	GCG135AC;AL's:
						TOURS TOUR MANAGERY CACCERACY TOUR CONTROLL COCCACCOCC AGGAGACA GCCCACCAC	GACCTGTTT	COCCEACCOCC	ACCCAGAGGA	CCCCCAACAC

CASTISANCE GGAGATTARC TACGARGAGA CASTISANCA GGAGATTARC TYTCAAAAA CCTTTAACAA	GGAGATTAAC	COCTGAACCA	TACGAGGAGA	CCTAACCGCA					•	
TACCCCCTAG CTTTCAAGGT GCGTCCCGCG CTCCACCCCG TACCCGCTCCCC AACGACGCGC TCCTCCTGAA ACTCGGGCTG CGCCTTTGGC	TCCTCCTGAA	AACGACGCGC	AGCGCTCGCC	TACCGCACTT	CTCGACGCCG	GCOTCCCGCG	CTTTCARGGT	TACCCCTAG	10607	
I DODI AMENERANE GAMENICA CGCARGGCGC GAGCTGCCKP, ATRICICETRIA TEGEGAGCGG ATRICICECTO AGGAGGACTE TOAGCCCGAG GCGCAALCEG	AGGAGGACTT	THECTOCOCO	TCGCGACCCG	ATRICICATIONA	CACCTOCAX	CGCAGGCGC	GAAAGITICCA	ATTENDED	10001	
			100000000000000000000000000000000000000	בוניהריווני	ACTAINCE	TEGACTICGE	ACTUOCOGO GETTEECALG TEGACTICSE ACTAINCIDEA EICUCANIC ACCASSOS CONTRACTOR OF THE STATE OF THE ST	ACTOCCOOLO		

TEMECESCAC CCANDOSTIC ACCITIANCES TOATACGEST CAGGESTACE TECETICARY GAACCTRITT COGRECOCG ACCIDANCE ACTECICALE ACTECICALE TECETICACE ACTECICALE TECETICACE ACTECICALE TECETICACE ACTECICALE ACTECACION ACTECICALE ACTECICALE ACTECACION A

CCCCCANCA COGOCTICCTIC

CONTRACTICE CECECOCICE CACTEGORGO COCCOUNTY RETAINED THE CONTRACT CONTRACT CONTRACT CAMBINITY COMMITTEE CO	CCACCIOCOS ACCETICIDOS COCASCAGAS GOTGGCTATA GAACTGATAC ATCTCTATAS CTTTATAAG GCGCTCGAGC AAAACCCAAA TAGAAKKIG CCACCIOCAGC ACCACGATA ATCATACGAC AAAAAAC CACCACCACA TATAAAAAC CACCACCACA CACCACACA ATTAGATATA ATCATACGAC	CTCATGGGG AGCTGTTCCT TATAGTGCA CACAGTAGAG ACAAGGATG; ATTCACAGATA GCGCTGCTA ACATAGTAGA GCCGGAGGGC CGCTGGCTGC GAGTAGCGC AGCTGTTCCT TATAGTACATCT CGGGCTCCCG GCGACGACG ACATAGTAGAGGA ATATCACGTC GTGTCGTCCC TGTTACTCCG AAATCATCT CGGGCTCCCG GCGACGACG
CCTCTAATT	GCCCTCCAG	ACATAGTAG TGTATCATC
SCACTTOOT	CTTTGTANGC	CCCTCCTAA
ATCCTCTCT C	ATCTUTATION OF	ATTCACCIAT (
CCATTGGCGT	GGACTGATTEC CCTGACTATG	ACAMPGARDE TGTTRCTCCG
CCCCCCACCT	GGTGGCTATA	CACAGEAGE
CACCITICGCOG	CIRCINGAGGA	TATAGTGCAG ATATCACGTC
COCOCOCOCA	ACCETIONO	ACCIVITICCT TCGACAAGGA
CCERRENCE	CCACOTOCOT	CTCATGGGGG
11001	11101	11201

24/144

		******								
11301	TCCATTICAL	AAACATCCTG	CAGAINTATAG	TESTACES						TTAGCCTCGG
	ACCTARACTA	TFICTAGGAC	CTCTCICIANC	ACHTACHTACT	כונארד ארבר צור	א ידיבאניניניאני	Terreconn	GCCANTAGATIG A	ATANGGTACG	AATKCCCCC
11401	CAACTITIAC	GCCCGCAAGA	TATACCATAC	CCCTTACCTT		ACCIACIONA	CATCCACACG	TTCTACATOC (	<b>CCATCCCCCT</b>	CAACTITICT .
	GTTCAAAATG	COGCGTICT	ATATECTATE	GCCAATCCAA	ממינואוריותיד	TITITICATET	CTACCTCCCC	AAGATTSTACG (	COTACCCCOA	כידוניכאכטי י
11501	ACCITICACCO	ACCACCTOGG	CGTTTATCCC	AACGACACA	TICK WARE		A SCCGGGGGG	GCGAGCTCAG	CONCCOCCIAG	CTSATTSCACA
	TOCHACTEGE	TOCTOGACCC	GCAANTARCO	THICTCGCGT	ACCEPTATE	מכאנדנה מניאכ	אימפכבמונים	COCTCGAGTC	פכיוספכתכים	GACTACOTUT
11601	GCCTGCAAAG	<b>GOCCCTGGCT</b>	GOCATORICA	GCCCCCATAG	ACAGCCCAAG	TCCTACTTR	ACGCGGGGGG	TGACCTGCCC	<b>TOGGCCCCAA</b>	GCCCAACTACGC
	COGACOTTAC	CCGGGACCGA	CCGNACCCGT	CACCICACTATO	TUTCCOCCTC	AGGATGANAC	TOCOCCCOCG	ACTIGICACOCC	ACCCCGGGGTT	COOCLOCOC:
11701	CCTGGAGGCA	acteoocco	GACCTURGCT	CACCATAGEA	בבבנונוננוננונ	CTGGCAACGT	COCCACCONG	GACCANTATO	ACCAGGACGA	TGACTACGAG
	COACCTOCGF	CCACCCCCCC	CTCCACCCCA	CCCCCALLUT	2020202020	GACCGTTGCA	DUCCECCEAC	CTCCTTATAC	CTOCT	ACTICATICCTIC
									Pstl	
11801	CCAGAGGACG		ACCUSTICATE	THETSARCA		GALGCAACTG			CTGCAGADCC	AGCCGTCCC9:
	GENERACTICS	CECTCATGAT	TCGCCACTAC	AMGACTAGE	CTACTACGTT	CTGGGTTTCCC	TGGGCCGX;CA	כפכנכסככסכ	GACGICTCGG	TCGGCAGGC
11901	CCTTAACTCC	ACCORDODACT	· occocchoor	CATCGACCGC	ATCATCTCCC	THACTRACIANG	CAATCCTVIAC	OCCULIOCOCC	AGCAGCCGCA	GCCANCCIA:
	GGAATTTGAGG	TOCCTOCTGA	CCCCCCTCCA	GTACCTGGCG	TAGTACAGCG	ACTGACGCGC	GTTAGGM:10	COCANGOCCG	restedeest	CCCCTTOCC.
							Pres			
12001	CTCTCCCCAA	TICTOGAAGE	: OCTOPICCEO	GCGCGCGCAA	ACCCCACGCA	CGAGANGGTG		TAMACCICCIT	GCCCGAAAC	AGGCCATC
	GAGAGGCGTT			COCOCHOTIL	TOCOGNOCOT	GCTCTTCCAC	GACCGCTAGC	ATTTOCCCCA	CCCCCTTTG	TCCCUGTAR
12101	OCCCCACOA		GICTACGACG	CACTGCTTCA	GCGCCTCGCT	CCTTACAACA	GCGGCAALGT	GCAGACCAAC	CTUSACCOCC	TOTOGOGGA
	CCOOCCIOCT		: CAGATGCTGC	GCGACGANGT	CCCCCCCCC	GCANTISTINIT	CGCCGTTGCA	corcrastric	GACCTGGCCG	ACCACCCCC"
12201	TGTGCGCGAG	occorococ	: AGCGTGAGCG	CCCCAGCAG	CAGGGCMCC	TOCOCTUCAL	CASTROCACTA	AACCCCTTCC	TGAGFACACA	GCCCCCCAN'
	ACACGCGCTC		* TEGENETICOC		GTCCCCTTOG	ACCCGAGGTA	CCAACGTGAT	TTGCGGAAGG	ACTCATGTOF	COGCICOSTE
12101	GHOCKOCOO	GACAGGAGGA	CTACACCAAC	TTYGICAGEG	CACTGCGACT	ANTGGTGACT	GAGACACCGC	AAACTCACGT	GTACCAGTCT	GOCCCAGACT
	CACOOCOCCC				CTCACCCCTA	TTACCACTGA	CTCTGTOOCG	TITICACTOCA	CATOGICAGA	CCCGGTCTGA
				_						
12401	Andrew	GRITTAGERAGA		CARGECTEC AGACTETAAA	CCTGAGCCAG	CCTGAGCCAG GCTTTCAAAA	ACTTGCAGGG	CONCROCCC	GRECEOSCIC	CCACAGGGGA
	TAMMANGGE			TUTORICATET	GGACTCGATTC	CHANAGETETE	TGAACGTCCC	CCACACCCCC	CACOCCCGAG	GOTOTOCOGCT
12501	CCCCCCCACC	GIGICIAGET	P TOCTGACGCC	CAACTEGERE	CTGTTAXTTGC	TYSCTANTAGE	מיתינדדתאכם	GACACTOCCA	OCUMENCEC	GGACACATAC
	GOCOCCICO	CACAGATCGA	ACGACTOCOS	CITICAGCGCG	מאכאאכמאכמ	ACGATTATCG	CTRTANGTESC	CTCTCACCGT	CGCACAGGGC	CCTGTGTATG
12601	CTAGGTCACT	TOCHOACACT	r GTACCGCGNS	<b>GCCATAGNTC</b>	AGGRECATOT	CHALCAGUAT	ACTITICCARG	ACATTACAAG	TGTCAGCCOC	GCGCTNGAGC
	GATCCAGTGA				TCCGCGTACA	CCTCCTCGTA	TUMAGGICC	TCTAATIGITIC	ACAGTCOGCG	COCCYCCCC
								7	Pmai	
12701	ADGACCACAC	: OCCCACCTC	S GAGGCAACTC	TAAACTACCT	CHITACTANG	CHACHOLAGA	AGATOCOCTO	GTTGCACAGT	OFFICEACAGE TENNACAGES	ACCAGGGAGGG
	recreated	CCCGTCGGAC	crecomitan	ATTIMINATION	רכאכיויניורי	ויכנואיכפובו.	TUTANKARAG	CAACGTGTCA	ANTINGREGE	TCCTCCTK(%)
12801	CATTTOCOC	: TACCTRICAGE	C AGAGGGGTGAG	CCTTMCCTG	היהההדדות		כאמכנידנהמים		CTRICACATICA CCRCCCCCAA	CATCGAACCK
	GTNANACGCG	3 ATGCACGTCG	ם דכותיתכתכיוכ	CGAATTCGAC	TACTACKTING	CCCATTCCGG	מוכנאנאניניני	GACCTIGTACT GGCGCGCGTT	GGCGCGCGTT	GTACCTTGGC
·.										
										•

Figure 15H

toort	CATT ATTERDED	ייידראאאיינים נ	CACCOUPTANT	AACCERCETAA	TYX:ACTACTT (	ניכעשנגייני כ	מבנטיבנים א		TITCACCAAT	CCCATCTTCA
10001	-				ACTIVIATION (	רהדאינידאני (	לטטלמטלאנד.	WESCHONT !	MAGTGGTTA	CYCTACAACT
13001	-		CCTOSTITUTE	אכאכיני אינאני	ATTECHACITES (	CUTCACERTA !	ACGATGGATT		GACATAGACG	ACACACATA
	-		GGACCAAAGA	אישראייכניניני	TAAGCTINCTAC (	CHARTECUTAL 1	TGCTACCTAR	SACCCTG	CTOTATCTGC	TOTOCCACAA
							± <b>¾</b>	HindRD		
12101	THURSDAY.	COCCACACOC	TGCTAGAGTT	מבאתבשנענ	מאקיבאנאיכאיפ	אתתתתתונד נ	CICCIAAACGAA AGCITICCOCA	AGCTTCCCCA (		CHIGHCGGAIL
10161	AAGGGGCGTT					אנהנשכטנוניץ (	CUCHTICCT	TCGAAGGCGT	CCGGFTCGFC	GNACAGGCT'.
					) lineliii					
1921	CTAGGGGGTO	COCCCCOCO	OTCAGATOCT ACTACCCCAT		TRECANSTT GATAGREETET			CTCGCACCAC	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CTOCTORGO:
1	GATCCGCGAC	ОССОВОВСОС	CAGTCTACGA		AACCTTCCAA	CTATCCCAGA	GNATGGTCGT	CAGCOTGOTG	0000000000	CACCACCC
			Pst	1						
12201	ANTOROGERA	CETAAACAAC	TCGCTGC17GC	אשכננטכאטננ	CGAMANAMIC	כתמכבתכנינפ	CATTTCCCAA		GAGAGCCTAG	TOGACAANIAT
1001			AGCGACGACG	TOTALCENCIC	GCTTTTTTG	מאכניםאמניככ	GTAMGGCTT	GITGCCCTAT	CICICOGAIC	ACCTINITION
וטאנו	CACTACATOR		CCCAGGAGCA	CAGGGACGTG	CCNARCTICC	CUCCUACCAC	COSTCOTCAN		GTCAGCGGG	rendencina
1010	CTCATCTACC		OCGNECTICAT	GTCCCTGCAC	COUNTRY TO STATE	CONTRACTOR	GOCAGCAGTT	recerected	CAGTCCCCCC	AGACCACACT
10301	CACTACTOR		CCACACCACC	GTCCTGGATT	TECHNICATAG	TRECARCECE	TTTGCGCACC	TTCCCCCCAB	GCTGGGGAGA	ATCTTTANA
TACCT	CHOCHENTAG	TGAGCCGTCT	GCTGTCGTCG	CAGGACCTAA	ACCCICCATC	ACCCIPICAGE	<b>NANCYZCTK</b> 25	AAGCGGGGTC	CGACCCCTCT	TACANASTIT
,		Charleton	SARTSARABA	CHENTRANGE	CCATTACACC	CACACATTAGAT	TITCHWITAT	TCCCCTTAGT	ATGCGGCGCG	CCCCCATCTA
13601	ANAGAMAGA	GCA15616CA	The second	CACHTACTUCE	GETACTORGE	CTCCCAACCA	ANACAACATA	ACCCCANTCA	TACCCCCCCCC	GCCGCTACAT
	TITILITE	COTAL TALL		2210000		المحدوديوروي		CCCTTCGATG	CTCCCCTGGA	CCCCCCTTT
13701	TCACCAACCT	כבוכבובכבו	CCTACCACAG		ואיז אלאינאיז		CCACCCAAGA	COSTABILITAL		GGCGGCM
	ACTCCTTCCA	CCACCACCA	GGATGCTCTC	ACACCACTCG	כפרכנייור	٨٠٠٨٠٠٨	Concerno			
		Kori								
13801	proceed	GGTACCTGC	<b>GCCTACCO</b>	GECHGNANCA		CITCITICACITAC	CCACCCCTAT	TEGACACCAC	CCGIGITATION	
		CCATGGACGC	COGATOCCCC	CCCTCTTIGE	CCTAGGCAAT	GACIACTICIAAC	CCTCCCCATA	ACCTGTGGTG	CCCACACATO	
13901		GGATGTGGCA	TECETIFIACT	ACCAGAACGA	CCACAGGAAC	THICHGACCA	COGTEATHEA	ANCANTONO	TACAGCCCGG	
			ACCCACTAGA	TESTETRECT	<b>GENETICOTITO</b>	AAAGACTGGT	CCCAGTAAGT	THETTACTE	ATGICOGOCC	
14001	_		ACGACCGGTC	GCACTRAGAGE	CHICKLACCTRIA	AAACCATCCT	CCATACCAAC		TCAACGAGTT	
	_		TGCTCGCCAG	נינידפאככככה	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	TITCHTACK	CCTATCCTTC	TACCCITTIAC	ACTIGGICAA	
14101			GATOCTOTO	COCTTGCCTA	CTAAGGACAA	Transmissions	CTGAAATACG			
4	TTATTCAAA				GATTCCTVTT	ACTICCACTTC	GACTITATIGG	TCACCCACCT	CAAGTGCGAC	cocrecet
					Paul			•		
14701	A COMPLETE	GACCATGACC	ATAGACCITA		TRANCANCIC GATCHTONA	CACTACTTCA		AARTGROCAG ACAGAACOGG		
10271	TERTENGO				CTACA:ACK:TC	CHICATGAACT	THEACCEGNE	<b>POTCTFIGGG</b>	CAACACCTTT	
14161		_		GGRETTING.	CCCMCACTR	CFINCHTACTICAT	CCCTVAXXCTA			
200		_			CKKICAGITIAC	にんにんんにんのすん	CGGACCCCAT	ATATOTHROC		
14401		CACCATCCCC	CONSCINCT	ACCCACACACC	GCCTV:AAC:AA	CHICHICACC				
					COCACTECITY	GANCANCCCG	TAGGCCTTCG	CCGTTCCCAA	CONCINCICCO	, AVATCCTAGE

Figure 15I

## PMRKAdSgag MER682

14501	CTTACCATOA	TOTAGAGGGT	CCTAACATTC	CCCCACTURE	CGATTGTAGAC	מנבווענינעט	CCACCTTGA	AGATGACACC	CANCAGGGCG	COCHOTOGC
	CGNTGCTACT		CCATTGTANG							כככבעכינונו
14601	AGGIGGGCAGC	AACAGCAGTG	CEANTAGE CEC	ההאאקאקאק הנידיורידיוני	TECANCISTS:	באנאינאינט באנט	AATGCAGGCG	CACCTCCTGT	TCAACGATCA	TOCCATTORN:
14701	GCCGACACCT		CACCTCACGAG				מ: זוא:כניו דכ	CCCCTCCCCA	ACCCOAGGTC	GAGAAGCCTY
	CCCCTGTGGA	AACCGTGTGC	CCGACTCCTC	Treacacana	reconcration	TCCCCTACTT	כניענממנטים	GACGACGCGF	1GGGCTCCAG	CTCTTCGGW
								Charles or a consistent	The state of the s	
14801	AGAAGAAACC ACTIN TINGG	CONGRETE	CCCCTGACAG	TCCTCTCTT	CHARLECON	ATCITICS ATT		GTCGTGGAAG	TOCOTCATO	CGTCGACCAT
	¥.									•
14901	CCTTCCATAC	MCTACCCC	ACCUTCACAC	COCHATECUC	TCATGGACC	TYCTTRICAC	TCCTGACGTA	ACCTGCGCCT	COCHOCAGOT	CTACTROTOR:
1	GGAACGTATG		TOCOACTOTO	GCCTTACOCC	AGTACCTINGG	ACTANACETE	AGGACTICAL	TOGACOCCGA	<b>OCCITODICCA</b>	GATGACCACIC
15001	TTGCCAGACA	TOATGCAAGA	CCCCGTTGACC	TYCCGCTCCA	CGCGCCCACAT	CASCAACTIT	CCGGTNGTVG	GCCCCCAGCT	ornacceata	CACTCCAAGA
•	AACGGTCTGF	ACTACGLICT	OCCCACTICO	ANGGCGAGGT	GUGCOCITCIA	GTCGTTGAM	GIXCLACCACC	CGCGGCTCGA	CAACGGGCAC	GTGAGGTTCT
										Asci
15101	OCTICIACAA	COACCAGOCC	GICTACTOCC	AACTCATCCG	CCAGITTACC	TCTCTGACCC	ACCTICITICAL	TCCCTTTCCC	GAGAACCAGA	THIROCOCITY
	CGAAGATGTT	GCTOOTCCOO	CAGATCIACICC	TTGAGTTAGGC	CCTCANATGG	AGAGACTGGG	TOCACAGGIT	ACCGAAAGGG	CICTIONICI	AAAACCUCC#*
1	-								THE BUREAU STREET	ACTION
15201	CCCGCCAGCC	CCCACCATCA	CCACCCATCAG	ACTITITICAN	GGACGAGAGT	GTCTAGTGCC	CTOCCATOCC	CACCCOTTCT	CCTAGCCTCC	TCAGCTCCCT
16301	CHONCANTER			Market Mark	TITTACABOSCI	CTREGGLATA		GCGTCCTATC	CAGCCCCCACT	TTTTGAGCAA
TOCCT			TOCOOCCIOG	ACCIONOCATOC	MATICITICOS	CHACCCCTAT	CAGAGCGGCG	COCACCIATAG	CTCGGCGTCA	AAAACTICITT
15401	GCATICALAT		CCCAGCAATA	ACACAGGCTG	CANCIGOGO	TTCCCAAGCA	AGATGTTTGG	COCOCCCANG	AAGCGCTCCG	ACCAACACCO .
	CUTACAGGTA		GGGTCGTTAT	TGTGTCCGAC	CCCOGACGCG	ACCEPTO	TCTACANACC	GCCCCGGTIC	TTCGCGAGGC	TOCT TOTAL
15501	Agracecare	COCOGOCACT	ACCOCOCOCO	CTRACEGEC	CACAAACGCG	GCCGCACTOG	פרהכתכריתככ	GTCGATGACO	CCATCGACGC	CONCERNICA:
	TCACCCCCAC	GCCCCCTGA	1000000000	פשככנוכפכנוכ	GROTTIGCGC	COCCUTCACC	CCCCTCCTCC	CAGCTACTOC	GGTAGCTGCG	CCACCACCT
15601	GAGGCGCGCA	ACTACACGCC	CACCCCCCCA	CCAGTGTTCA	CACTRICACGC	GOCCATTICAG	ACCEPTANCE	GCTGAGCCCC	OCCCTATOCT	AAAATGAACA
	CTCCGCGCGC	TGATGTGCGG	GTCCCCCCCT	GGTCACAGGT	GICACCTIGCO	CCGGTMGTC	TCCCACCACG	CCCCTCGGGC	CCCCATACGA	THE ACTION
15701	GACCOCCICAG	GCGCGTAGCA	CGTCCCCACC	CCCCCCCACC	CCCCACTCCC	הנכניאסאנפ	בישכמשכשנ	CCTGCTTAAC	CGCGCACCTC	GCACCTATACG
	CTGCCGCCTC	COCGCATCGT	acacceated	COCCOCCIGG	CCCCTGACGG	COCCITOCOC	93364336339	GCACCAATTG	OCCCGTGCAG	certacecaac
	•	Sfil								
15801	ACOGGGGGGCC	ATCCGGCCG	CTCGAAGGCT	GOCCOCOROCE	ATTRITICACTO	TRECORCICCAG	GTCCAGCCGA	CGAGCGGCCG	CCCCAGCAGC	COCOCCATT
	160000000	**************************************	GAGCTTCCGA	CCCCCCCCCA	TAACAGTY:AC	ACTAGGGGGTC	CAGGTCCCCT	<b>GCTCGCCGGC</b>	GGCGTCGTCG	GCGCCGGTTAA
15901	AGRECTATEA	CTCAGGGTCG	CAGOGGGAAC	GTGTATTCGG	TOCOCGACTC	CONTRACTOR	CTRICGCGFTGC	CCGTGCGCAC	ددعددددده	COCAACTARA
	TCACGATACT	· CAGTCCCAGC	GICCCCGTTG	CACATAMCCC	ACGCGCTCAG	CCANTERICUS	GACCCTX:ACG	COCACOCOTO	2000000000	מכפדואיאדגיד
16001	TTGCAAGAAA	NAACTACTTA	GACTCGTACT	CHICTATCIA	TCCAGCGACG	CONTRACTOR	ACGANGCTAT	GTCCAAGCGC	NAMATCANAG	ANGAGATRICT
	MACGITICITY	TITICATICAAT	CTGAGCATGA	CAACATACAT	AGGTCGCCGC	CARCCAGGGT	TGCTTCGATA	CAGGITICGCG	TTTTAGTTTC	THETETACGA

Figure 15J

ACATTGATAA AACATCTTAC CTTCTGTAGT TGAAACGCAG AGACCTXXXC GCTGTGCCGA GCGCGXXCAA GTACCCTTTG ACCGTTCTAT COCCURRCAT TATACCASA GROBACCASC GAGSCANAGO GCCACGGCCC TANCXCTCCT TCTTACCTCG CATCCTCCCC GTACCOGGCG GTGCCGGACT GCCGGCGGTA GAGACTICCGA GTTCTTGCAG CTCTCACGCT GOCCTANAGO GATTEGCCGC CTAACCCCC ATCITACCO TACCATURGET COCCGGGACT CECCEGITAR PICTICACE GCGCCCTGA GGTTGCCTCA CCAACGGAGT TCAGCCCCCC ACTCGGGGG TAGCACCGAT CCCGATTICC CCAGAAACAC CAAGAACTT NOCAGGICAN CTGGACGAAC TCGTCCGGTT **OCCCGTANCA** ECECTEDADE GRANGECTITI EXECCTATIC CIGIAGGACE GEANGGION CETECTECEG INGGINGIO GAICGGATIT COCOCATIGI TITITICACAA AACGCTGG:> GNANGATICAT ATCGTGGCT TROCGACC CTTTCTACT AFFECACTER TEGEEGEGE ACTITISCATE TITIBACETED CAACACACAT CACACECTIF CATAGRAAAC AACGINGGCA CCGGAACGIC COCGICICIG IGACIAAITI TICITICAACG TACACCITIT TAGITITAIT ITICAGACCI TACCTICTOTO TITTOCAGGGG CACGGCCTOA **ANAGICTOCA** CONOCAGOTO TOCCCACCTA CAAACCCCAA CCCASIONACEC CETCECCAREC CCGTOCTARC GCCACGACCG TAAGGTGACT AGCGGCGCCG CTAGCCTANA GCACOTCGAC AAGCAGGTGG **AMCOTOCOC** GTTICGCGTT ACCCCCCGGCT TOCOCOCCOA GENETIFICA CCAGGICATE GCGCCGGAAA TETATGOTO: CCCGAAGAAG GAAGAAAAA ATTACAAYCT ETGAAAACTA AAGCGGGTCA AAAGAAAAA THICHITE AAAACCTICTT CHECKINITATIC ATGAGGINATA COLOGACIAG GACCTOCITIO AAATTTTCGG ATCTAGGAAG GTAACGCGGA **GCAGCOGTCO** TTTAAAAGCC CHECENTIFICE COSTACCEDED ATTECCIANCIA AGANTOCIACE GTAGGRAGGIO CANGGEOGOCO ATCARATA CACAGAGGC ATCGAGACAC GICACCITITC CAGCIGCCCA TACTCCACAT GCCGCTGCTC ANCCCARCAC TOCCACCCAC ACCGROGOTO GCGCCCAATC GTCCAAACTG ACCCGTCGAT TACATCCTTC CATTGCGCCT GINCOACCOT THUGGCCCAGT OCCITACGCGC COCCATAGIA COCOGAGGAA CCCARCATICG TECTECOTEC TRABACCACG ACCATTGTEG COCGATGGTG COCTECTAGE CRYATISTICS OCCUPANCET OCCUCATORY ATCTCCAAAA COCCOCCAGCG AGGGCGGCT AAALTGGAG TCTGGTGACT AGACCACTGA GGAACCTYNG CTYNGARCCCG AGGICCGCGT TCCANGCGCA CACGETETISCC GOGCITICATE CITIVATERING TAANVATAKAA GOCCITICAT CGACGACTEC GIGICICCCG CACTOGNAM GCGCAGAGAC ACTGATTANA AACAAGTTAC DAGCAACTAC CCGACCCCGA ACCACCACTG GAACCCGCCG TCCCCCCTA TFTCCCCCTC AGCACCAGTA TTRECACCGC GRGATORITCA TECHTGOTCAT ANCIGOTGGCG CTCTACTAGA CTTATACGGG CICCITICATO GOCTGCGGCT TROTGCTGAC CITTGAGCGGC CCTTRGACCC GACCTCGGGC CONCERCENCY CEACOCCOXC GCANTITICITG GAGATACCTIC CANTATRICCC CCCTACCAC CHRICACOCTA CITYTHICKAN RICAANGOTA COCTOCC! AT INDECACTOR CRANCIFICACI CITICANTOTTO GCTCACATAC دنسيدنديجي THEATGCCUE GGCGOTCOCO CGATGACCOC TYSCONCASC CGTCCAAGAC GCTACTGCCC GCTCCACCCG CACCTACAAG GREGALITIG CCTACGGAAA GCGGCATAAG GACATGCTTGG CCACCITGAC GACGINIGAT (ACCIONOCIT) THETAGAATE GAAGACATEA TOCCTCGCGA AGRIAGACAGO ACCCTGGTGC GTCCCACCGT CAGCGTGGCA **GETTING GASCOG** TCCCAACAAA ACCACOCCO CGAACOTOGC AGGITTCTTT NATCACCCT THINCTORCA CACTACCAGT CCCCCACCCC 3000000000 Greensacce ecoeconoco GGCCTTGCAG **GCTTGCACCG** TOCCACCTOC ANGTICTATION MAGTACGGC ACTURADAT STUTTESANA TCACCTTCTA CAGAACCTTT CCACCCCCTC TTCAGATACC COCCOCCTCT AGATACCGOG DETACAMETIC AGENTIFIACO COCCUERDAGO TTGCATCCOT TGTANCTATT CACCTOCCOC CACCACCOGC **GOCAACCTCC** ACCOTOGACG ATCCCCCCCCT TACOGCOCCA CCGTTCCAGG CCCAGAAGAC GOCTUTTOR ACCOAGCCCT AACTGCTGCT TCAGANATGC TOCTGCCCGC TTGACGACGA CITECAGCAGG CACCOCCCTT COCTROOTCC GCGNACCAGG COCNOCACOC GTOCCCGGAA CACCTACCGC GTGCGCNGG ATATOGCCCT **acorcorace** OCCUPACAG CCCGCACGTC DCCGCTCCCCC COCCACCOCC 000000000 CCCCCCCCCCC STOCATOOCO CACGCGTCCC **GACGTCCTCC** recedence ACCOCCAGCG CTACTACTTO CGTOCTOCCA COMPCCICTC GCTCGCGGAG COTCCAGTAG GATGATGAAC CCACCACCGT E 17501 17201 17301 17101 17001 17401 16901 16601 16701 16801 16401 16501 16201 16301 16101

Figure 15K

	Confly										
	***										
7601	TCCCCACCAG		<b>GGT</b> GGCGCCT	TCAGCTGGGG						GCA(4) AN(A.7	
	AGCCGTOGTC	GITATACTCG	CCACCGCCCA	<b>AGTEGACTEC</b>	פאהתיאות אין	TYSCCGTAAT	TITTAMAGE.			cercerrer:	
7701	CTCCAACAGC	ACCACACACTC	AGATYPETTANS	CHARACTE	ANNUNUNUNA	ATTITICABEA	AAACKFTCKTA (	GATGGCCTCG (		TAGCGGGGTT:	
	GACCTTOTCO	reoretees	TCTACGACTC	CCTATTCAAC	E	TANAGGTTIGE	TTTCCACCAT	CTACCOGACC (	CCACACCGTA	ATCCCCCCN:	
									O CONTRACTOR OF THE PERSON OF	B. A. P. P. C. P. P. C. P. P. C. P. C. P. C. P. C. P. C. P. P. P. C. P.	
7801	GTOCACCTOG	CCAACCAGGC	ACTCCAMAT	AAGATTAAGA							
	CACCTGGACC	COTTACTOCG	TCACCITIFITA	TICTAATIGE	CATTECTIAACT	אנאטטטטטטטע	האינותאכאונ			TOTACAGI	
7901	CAGAGGGGCG	TOCCCAAAG	COPRICACION	CCGACAGGGA	AGAMACTECTS	ייין אייר האיי	オルシカクロカロウに		CAGGAGGCAC	TANAGEDANT:	
	GICTCCCCGC	ACCGCTTTTC	GCAGGCGCG	GGCTGTCCCT	TETTTICACIAC	CACTGCGTTT	ATCTGCTCGG	AGGGAGCATG	CICCICCGIO	ATTECTIC	
18001	CCTOCCCACC	ACCCGTCCCA	TCCCCCCAT	TOCOCOCCAT COCTACTOGA	הוהאידיהוה	אטנאנאנאככ	COTAACGCTG	GACCTICCCTC	CCCCCCCCGA	CACT: AGCA:	
	GCACGGGTGG	TOGGCAGGGT		AGCOCOGGTA . CCGATGGCCT	CACCIACCCCC	TCCTCTCTCC	GCATTGCGAC	CTGGACGGAG	GCGCCCCCT Prof	GINICOTOCT:	
									***********		
10181	AAACCTGTGC	TOCCAGGCCC		GTTGTANCCC			CCCCCCCCCCC		OCCUPACGING	COCCCCTAG	
	TTTOCACACG	ACGGICCOGG	CTGGCGCCAA	CAACATTKAG	CAGGATCGGC	CCCCAGGGAC	ටලපටඉටඉටඉ	GOTCUCCAGG	COCTAGCAAC	GCCGGCCATC	
18201	CCAGTOCCA	CTGCCANGC	ACACTGAACA	CCATACCTICCE	TCTOCOGGTG	CANTECETTA	ARCOCCGARG	ATGCTTCTGA	TAGCTAACGT	GICCITATGRO	
	GOTCACCGFF		TOTOACTIGE	CGTAGCACCC	MGACCCCCAC	GTTAGOSACT	TCCCCCCTVC	TACCAAGACT	ATCGATTGCA	CACCATACA .	
19101	Tercatorat	GCOTCCATOT	CCCCCCAGA	CHARCTECTO	ACCCCCCCCC	CCCCCCCTTT	CCAMGATYSC	PACCCCTTCO	ANGATGCCCC	ACTCCTT A	
	ACAGTACATA	_		CCTCGACGAC	TECHEGOGGC	CCCCCCCCCANA	<b>GGTTCTACCG</b>	ATGGGGAAGC	TACTACGOCG	TCACCAGA: r	
19401	CATACACATE	PCOGGCCAGG	ACCITCGGA	GTACCTGAGC	CCCARGCTGG	TOCANTITUE	CCGCGCCACC	GAGACGTACT	TCACCCTCAA	TANCAAGTIT	
,	GTACCTOTAG				CUCCUCCOVCC	ACCTUAAACG	CCCCCCCTCC	CTCTGCATGA	AGTEGGACTT	ATTICTTICADA	
10501	BERRRETTER		TACCELEGE	GREACTACAG	ACCORPACICA	CCCTTTCACG	CTGCGATTCA	TCCCTGTGGA	CCCTGAGGAT	ACTOCOTACT	
1000	TCTTIGGGGT	GCCACCCCCG			TOGGCAGGGT	COCAMICTOC	GACGCCAAGT	AGGGACACCT	GCCACTCCTA	TGACGCATGA	
18601	COTACARGG		CTAGCTV:TGG		TGTCCTGGAC	ATGGCTTCCA	CCTACTTICA	CATCCOCOGC	GTGCTGGACA	GCCCCTA-	
	GCATGITCCG				ACACGACCTG	TACCGANGGT	GCATGAAACT	GTAGGCGCCG	CACCACCTGF	CCCCGGGATI	
18701	TITTAAGCCC	TACTCTOCCA	CTGCCTACAA	COCCUTOCT	CCCMGRATE	CCCCAAATCC	TYGCGAATGG	CATCAACCTG	CTACTCCTCT	TGAAATAAN	
	ANAATTCGGG		GACCGATCTT	CCCCCACCCA	COCHICKY	COCCUTTAGE	AACCCTTACC	CTACTTCGAC	GATCACGACA	ACTIFICATION:	
18801	CTAGAAGAAG	ACCACCATCA	CAACCAAGAC	GAAGTACACT	AGCAACKTIGA	GUNGUNAAA	ACTEACGEAT		<b>CACCIFICATION</b>	GCTATAAATA	
	GATCTICITIC		GITOCITICAG	CPPCATCTGC	TURTTECACT	CGTCGTTTTT	TGAGTGCATA	AACCCGTCCG	CCCRATAAGA	CCATATTTAT	
18901	TTACAAAGGA	Ξ.	ATAGGTOTCG	AACCITCAAAC	ACCTANATAT	GCCGATAAAA	CATTICACC	TGAACCTCAA	ATACCAGNAT	CTCACTYGIA	
	AATGITTCCT	_	TATCCACACC	TICCAGITING	TGGATTTATA	COCCTATITI	GENNAGTTCIC	ACTTGGAGTT	TATECHETTA	GAGTICACCAT	
19001	CCANACAGNA	ATTAATCATO	CAGCTGGGAG	AGTCCTANAN	ANGACTACCC	CAATGAAACC	ATITACOGE	TCATATGCAA	AACCCACAAA	TGAAAATGG"	
	GCTTIGICITY	TAATTAGTAC	GTCGACCCIC	TCAGGATTTT	PICTGATOGG	GITTACTITIOS	TACAATGCCA	ACTATACGTT	TIGGGIGHT	ACTITION OCT	
19101	GOCCAAGGCA	TICTIGIAM	GCMCMMT	GGANAGCTAG	ANACTCAACT	CHANATGUAN	TPTFTCTCAA	CTACTGAGGC	AGCCGCAGGC	AATCCTCAT .	
) ! •	CCCOTTCCGT			_	TITCAGITICA	CCTTTACGIT	NAVACACIT	CATGACTCCG	recentee	TTACCACTAR	
19201	ACTITICACTICC	TAAAGTGGTA	TTGTACAGIG		TATAGAAAGG	CCAGACACTC	ATATTATA	ATATTITITA CATGCCCACT	ATTANGGANG	GTAACTCAC!:	
	TGAACTGAGG	ATTTCACCAT	AACATGTCAC		TICTACATET ATATETITIES GETETRINGS	GRITCINGTICAG	TATAMAGAAT GTACGGGTGA	GTACCCCTCA	TAATTCCTTC	CATTGAGTR:	

19301	ACAACTAATO							CTAATGTATT J	ACAACAGCAC (	CCCATTATA
¥	TCTTGATTAC	cccorretta	GATACCCCTT	הדנימיאדא						
8 6	CCACAMENCE	GCCCATTCG '	ATCCC: ACTTCC TAGCCTCAAC	TTACYDACAAC	TAGATTTGCA /	AGACACAAAAC A	ACAGAGCTITE O	CATACCACCT OTATOOTCOA 1	MACGARCTA	AGGTAACCAL
) 4	ATAGAACCAG		ATCTCCCAATC		CAGGTATGAT (	_				AACTICCAAA
-	TATCTIGGIC	CATGAAAAGA	TACACCITING	TCCGACAACT		-				TIGAME
•	TTACTOCTIT	CCACTOGGAG	GTCTGATTAA	-			-			TGCTACACAA
-	AATGACOMA	CONCOCCTIC	CACACTAATT	ATCTCTCA		-	_		CCCITITICE	ACCATOLLS.
•	TITICAGATA	AMATEANAT	AAGAGTTCASA	AATAATTITIG		-	_		CCHGTACTCC	AACATAGEE
_	NANAGICTAT	THITACTITA	THETENACET	TTATTAMANC	GGTACCTTTA	-	COCITICGACA	_	GA CAT GAGA	TIGINICA
_	<b>POTATITICE</b>	CEACAAGCTA	AAGTACAGTC	CITCCAACGT	AAAAATTTCT (		ACACCTACCIA		AAGCGAGTOG	100C1CCC'
	ACATAAACGG	OCTOTICGAT	TTCATGTCAG	GANGGETTOT.A	TTTTTWAGA	CTATTCGGTTT '	TCTCGATGCT		TICACICACC	ACT GAGAGO.
	OCTACTOGAC	TOCTACATTA	ACCTIOGNAC	ACCEPTAGICC	CTTGACTATA		CARCCENTIF		CCAATGCTGG	CCTGCGCTM
	CCATCACCTO	ACCIATOTAAT	TEGANCETEG	TGCGACCAGG	GANCTICATAT	ACCTGTTGCA	GITGOSTANA	ricercerce	COLLACGACC	GCACCCCATT
	COCTCAATGE	TOCTOGGCAA	TOGTOGOTAT	GIGCCCTTCC	ACATECAGET	GCCTCAGANG	TICTITICECA	TTAMMACCT	cerrencero	CCGGCTCAT
	GCGAGTTACA		ACCAGCGATA	CACGGGAAGG	TGTARGTCCA	COCAGICTIC	MAGNANCOGT	AATTTTTGGA	GGANGAGGAC	GGCCCGAGTA
					Pstl					
	Branch Branch	CHEST A BUTTLE	ACSCARCETATIC	TTAACATGGT	TCTVF.AGAGC	TCCCTAGGNA	ATGACCTAAG	COTTGACGGA	CCCACCATTA	AGTTTGATA:
	TOTAL	_	TCCTTCCTAC	AATTIGTACCA	AGACCTICTICG		TACTIGGATIC	CCAACTGCCT	COCTCCTAAT	TCAMCTAIN
		_	"HYPETER CAT	GCCCACAAC	ACCIOCUTOCA	CCCTTGAGGC	CATGCTTAGA	MCGACACCA	ACCACCACTC	CILLIANCO!
	GTAAACGGAA		AGAAGGGGTA	ceaeterte	TCXXCXCAGGT	GCCAACTCCG	GTACGAATCT	Trecretoer	TGCTGGTCAG	GAMITICE! :
	TATCTOCK	_	GCTCT/ACCCT	ATACCCCCCA	NCTACTACCAA	CONGCCCATA	TCCATCCCT	CCCGCAACTG	OCCOCITING	COCCACTORY
	ATAGAGAGG	_	CCACATOOCA	TATOGGCCGT	TEXCENTEGIT	GCACGGGTAT	ACCTACCCCA	GOCCOTTGAC	CCGCCGAAAG	GCGCCSACT.
	The second second		AAGGAAACCC	CATCACTGGG	CTCCGGCTAC	GACCCTTATT	ACACCTACTC	TOCCTCTATA	CCCTACCTAG	ATCCARCET
	COMMETCEC		TICCTTIGGG	GTAGTGACCC	GACCCCCATTS	CTGGGANTAN	TCTCCATCAG	ACCCACATAT	GGGATGGATC	TACCTICALA
	STATE OF THE STATE	_	AGANGCITCOC	CATTACCTIT	GACTETTETT	TCACCTCACC	TRECANTRAC	CCCCTGCTTA	CCCCCAACGA	GTTTCANNTE
	AATOGAGTIG	CHETCEANAT			CTCAGAAGAC	NUTCGACCOO	ACCGTTACTG	GCCCACCAAT	CCCCCTTCCT	CMACTITIAN
	AACTONICAG		GGCTFACAAC	GFTGCCCAGT	GTAACATGAC	CAMGACTUS	Trectitional	ANATOCTAGO	TNACTATANC	ATTYRICTION
	THEY				CATTIGTACTG	GTTTCTGACC	<b>AAGGACCATG</b>	TTTACCATCG	ATTICATATTIC	TWCCGATGE
	Was State Man		ACCTACAAGG	ACCOCATOTA	CICCINCITY	AGAMCTICC	AGCCCATGAG	CCGTCAGGTG	GTCCATCATA	CTANATACAA
	TCCCCAAGAT				GACCAAGAAA	TETTTEMOG	TCCCCTACTC	GCCACTCCAC	CACCTACTAT	CATHTATCT
	CONCERNA			ACACANCANC	TCTCSCATTING	TIGGCTACCT	TGCCCCCACC	ATCCCCCAAG	GACAGGCCTA	CCCTRACTION.
	CONC. INC. INC. INC.	r encryconer	AGGATGTGGT		AGACCTANAC	AACCGATGGA	ACCCCCCCCTCC	TACGCGCTTC	CTGTCCGGAT	GOGACCATTC
							Prod			
				A COLUMN	TEACTOR SALAR	AAACTTTACTT	TYSCATCOCA	CCCTTTGGCC	CATCCCATTC	TCCAGTAACY
	PROCECTATE	COCTINIAGE	GTTCTGGCGT						GTACGCTAAG	ACKERCATTICA

Figure ISM

	K F		t s	l P	St E	: E	<b>5</b> :	j	s <del>t</del>	<u> </u>	кħ	R F.	<b>B</b> H	F B	t s	ដ្ឋ
4	CCATCCACGA	CACGCCCTTI GTCCCCCAV	TCAMGATCT AGTTTCTAGA	CCCCCAGICS -	CCACTCAAGC GCTGAGTTC	AAAGCGTAC . TITICGCATGT	CACCATGAN	OAGCGCCAC CTCGCGGTG	CTTTCAATAA GAAAGTTATT	COCATCOCTA	TCACTCCAC/	GATAUACAGG CTATGTGTUT	GTTRICTCAGG CAACGAGTCC	TGACCGTCCC ACTYGCACGG	CGFAAGACTT	GTCTTCACG
Rami il	GAGGTURATO	TGTACCTGGG AC/ "YGACGC	AAAGCCATTG TTTCGGTAAC	TAGTCAATAC	TTCTGACCAG AAGACTGGTC	AAGTECACCC TTCAGGTGGG	ATCACAACCC TAGTGTTGGG	CAGCTTCCTO	ACTAGACACA TOATC ICTOT	GCTTCTGCCG CCAAGACGGC	CCACTTCANA	CGCGAGTTOC	GOTCCTCCGC	CATCAAAGG GTAGTTTTCC	AAGAACATGC	GGCCCCACCG
	CATCACTTIT	ATCGAAACCG TAGCTTTGGC	GCAGGAACTG CGTCCTTGAC	GCCTGCGCCA CGGACGCGGT	CCTTTCCCTT	AACGCTGGAA	ACTCCCA1GG TCAGGGTACC	AACAGCTCTA	AAAATAATGT TTTTATTACA	AMTCAMGO	CCCCCTCCAG	GCCCTOCOCO	TCCGCGTCCA	ACCOTAGEOS TOGCATCACC	CCCTTCNSAG CGGNAG I: TC	ACCACATTRE GGCCCCACCG TGCTCTAAAG CCGCGGTGGC
	ACCCCCT1ACA TGCGCGATCT	CHOCOCATOR	GCTCCAGTGA	ACACAAGCTC TSTYSTTCGAG	CTCTTTCAGE GAGANACTEG	ACCCCTCTAT TOCCGACATA	CTGGCCCCAA	COCANCEASO	ANANCATGTA TITITGTACAT	CCCCCTTTAA	ACAACCATCC TGTTGGTAGG	TGGGGCCTCC ACCCCGGAGG	CCAGATCAGA	TTCCACTCTCC AACGTGAGCG	CAGCCTTTGC	GGACATY TYPE CCTCTAGACG
	AACTICACACCCC	ACCIONAL COCA TUGICAGEOT	COCCCCATOC	AACAAAGAGG	AACATGCTAC TTGTACGATG	TCTTCCCCCG	CCTTTGCCAA	CACCCTGGGT	TCTCACTTGA ACAGTGAACT	THECCGTCTTS	AAACTCAGGG TTTGAGTCCG	AAGTCGCAGT TGGGGCCTCC TTCAGGGTCA ACCCCGAGG	COCTATTICATE GCGAGAAGAG	ACCEANACTE	AAAGCCACCT TTTCGGTGGA	
:	TCTCTACOTC AGAGATGCTAG	GAGGGACACA	AACAACAGCT	TTTCCAGCT AAAGCTCCCA	CGCACTCANA	CGCCATTCCT GCGGTAACGA	TTTCTCCACG AAAGAGGTGC	AGGTACARKE TECATOTEGG	CACTTCTTTT	ACCCCC ACCC	TRCTCCACTT ACTAGGTGAA	ECATATICTEG CETATAGAAC	CHARCEAGEA	COCOCACOSO	GATCHGCTTA CTAGACGAAT	GREGINGIAGO CAACAACOTTIS COTCAGRATT CAGCACOTIXE GTOGTFGAAC ACAACCACAA
	COSTITIOGA	CTTTRACTE	MOCMCATC	TCACAAGCGC ACTGTTCGCG	GCCTF3GAACC CGGACCTTFGG	TCCCCCCTAG ACCCCGCATC	CTGCTGCATG GACGACGTAC	AACAGTCCCC	TTAGGAGCGC AATCCTCGCG	GTCATTATTT	TOGTGTTTAG ACCACAMATE	GETECOCCCC	GTGGTGCACG CACCACGTGC	CCCAMAAGG	TAMAGECTT	
	ACAGACCTAGG TGTCTAGACC	TGTTTGAAGT	ATMAGAAGC	TOCCACCTA ACCCCTCGAT	CTACCGGAAA	GAGTCACTCC	GTGGACTATT	CTCCATCCTC	AGTECCEAGA TCACGCGTCT	ACACTCTCGG TGTGAGAGCC	OTTOCCATAC CAACGCTATG	GCCTTTACCA	TCAGCGCCCGG AGTCGCGGCC	TAGCTGCCTT	AGCCCCTGCA TCCCCCACCT	SEI GCCGGAAAAC TGATTGGCCG GACAGGCCGC CGGCCTTTTG ACTAACCGGC CTGTCCGGCG
	CCCCCCTCAG	CTTTAIGTTT GAAATACAAA	ACCCCACAAC TGCGGTGTTG	CCATATETET	CCCATCTGAC	GAMACTCATO	TCGGCCGCCT AGCCGGCGGA Korf	CCCATGOGIT	CCCCAGCCAC	<b>TITITATTICT AAAATAAACA</b>	GCAGGGACAC COTCCCTGTG	CATCACCAAC	TOGAACACTA	TCAACTTTGG AGTTGAAACC	GTTAGGATAC	SSI TGATTGGCCG GACAGGCCGC ACTAACCGGC CTGTCCGGCG
	TTATCTCCAT AATACAGGTA	OCCCACCCTT CGGGGGGAA	TCGGCCGGCA	TOGITICATOGO	CTCTGACCCC		OCCCOCCAAC CCCCOCCTTG	CITATIACCO GAATAATGGC	CCCCTACTT			GOCTGCGCAC CCGACGCGTG	GTTGCAGCAC	GCGAACGGAG		GCCGGAAAC
	21001	21101	21201	21301	21401	21501	21601	21701	21801	21901	22001	22101	22201	22301	22401	22501

Figure 15N

ATTENCATA ANGCITICGT TANATAGINI TACGNAGGIA	GREACETETH CHANGACITG CAGTGGAGAC GITTIVICTGW		CATCAGCGCG CXXXCAGCTT GTAGTCGCGC GCCCOTCGC 1		CCOGTGGGTT	AGANGGGCGC TCTTCCCGGG	CTCAGAAGGA	10CT1CCCCC	GATCATGGAG	CAGCITCCOTO GOGGCGAACT	AAAAGCAAGA CCAGGACAATT	GAAGCATCTG	CACCTATICT CACGGCGCT; GTGGATAAGA GTGGCGCCCA	TCGAACGOTC CTATCACATE  ACGAACGOTC GATACTEAL  EOFIT	Children of the Control of the Contr
CGRGCTCCTT GCACGRGGAA	ATCCTTCTAG TACGAACATC	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	GGTACTTGTC	TTCGCTGGGCCCG	TTCATTAGCA AACTAATCGT		CACATACACTA CACAA	ACCTCCTCCA TGCAGGAGGT		CACCTTCCCC	ACAGAGGATA	ACGTGCTGTT	CTACGAACGC GATGCTTGCG	GTGCCAGAGG	
ATTICAATCA	TGGGCTCTG ACCCGAGGAC	CAGCTGCAAC	TTATCCACGT AATAGGTGCA	CACTITICCOC	TTTCCCATCC AAACGGTACG	<b>GRECTER CONTROLL CONTROL CONT</b>	GCACCAGGGG	CAGAGACGAC		ACGCCCCTAC TGCGCCCATG	CTCAGTACCA	OTCOGAGACC CACCCTCTGC	TCAGCCTTGC AGTCGGAACO	CCATACTTRCCC GCATAAACGG	
CCACTGTAGG	במרוודכיותכ	TKATTANAST ACCACTTCCA	CTTTAGATCG GAAATCFAGC	ACCGTANTIT	GCTTACCTCC CGAATGGAGG	CTCTCTCTGAT	CCACACGCGC CCACACGCGC	GCGACGGGGA CGCTGCCCCT	GRECATTICE	GATGCCGCCA	ACGAGGACCG TCCTCCTGGC	CTACCTAGAT	ATAGCGGATT TATCGCCTAC		
			TEAAGTTEGE ACTTCAAGCG	CGCGTTCATY: GCCCAAGTAG	recaetratec Gegraacaeg	CCACCATTAC GGTGCTAATG	CCCCCCCCTC	CCTCCGCCGC	CTTCCCCACT	CCANATOCACC GCCACACATAR	AGCGAAGACG TCGCTTCTGC	COCATOGICGA	CHANGE AND COMP	CCGCCCCCCACT	
מנוסבתאבונים המאחאמנים במכנהקאמנים המאחאמנים	CHEAGRAGHS CMACACAAC GCGTCGCCAC GREATHG	COTCACAAAG GTCTTRITTRIC GCAGTGITTIC CAGAACAACG	CCCTCATCAN C	GCACACTCAG	ATTCAGCCGC TAAGTCGGCG	TCCTCXCTGT AGGACTGACA	ACCITCTATES TCCAGCTACC	صعدوددددد	COCTOCTCCT	TCCCCACCAC ACCCCTCCTC	AGGITITIGIA TCCAAAACAT	CCCCTCCTTT	GCAGCGATGT	CCACCCCAAC GCTCGGGTTG	
CHICHTEANC (	TCGATTTTAG (	CCCCCATCAT (		GACACGATCG	CCAGCAGAAG	THETETHTET	TCCCCCCCCCCCA AGGCGGCCGCCCCC	<b>GCTTTTTT</b> CO <b>CGAAAAAAC</b> C	CCACCAMOC	CCCTCTGAGT	AGCAGGACCC TCGTCCTGGG	AGTETEGGGG	THECOMORGE	ACCCATGTAC	
TRETAGACTE C	AAGCTCGCCT 1 TrcgAGCGGA 2	AGGAATC TCCTTAG		GAGGGTGCCT		GCGCCACATC	ANTOGCCAAA	COCCTCATOC	COCOCTCOO	CETAACEGEE	OTCATTATCO CACTAATAGC	ACCAGGAACA			
ATCTTGGCCT 1 TAGAACCGGA A	ACACTT	CAGGTACGCC	១៦	CCATCCCTT	4 E	< E	TCTT00GCGC AGAACCCGCG	CTCGATACGC	CCACCOCOTC	AGAAGGACAG	CCTCCTCCTT	CENCAGGCAA	GCGCCATTAT	ACCCCCAA	
22601	22701	22801	22901	23001	23101	23201	23301	23401	23501	23601	23701	23801	23901	24001	

Figure 150

## PMRKAdSgag MER6R2

24201	CCTCGCTCAA	CGAAGIICECA	MAATCTITG	AGGGTTTTTG	ACCENTACTAG	AAAAAAAAAAA	ANGRESTER CAAACTICTE GEAACAGGAA	GCANCAGGAA	ANCAGEGANA	ATGAMICTCA
	GCAGCCAGTT	GCTTCACGGT	TETTAGAAAC	TCCCAGAAAC	TRUCKETUCTE	TRUBURACC	GITTGCGAGA	CCTTGTCCTT	Trescent	TACTITICAGE
		•	Xhol							
24301	CTCTGGAGTG	THEOTOGRAC	Techacectea	CAACGCCCCCC	CTAGCCCTAC	TANANTECAG	CATCEAGGTC	ACCCACTTO	CCTACCCGGC	ACTIVACCTA
	GAGACCITCAC	AACCACCTTO	AGCTCCCACT	GTTGCCCCCC	GATTGGCATG	ATTTACCORC	GTARCTCCAG	TOCCTICABAC	GCATGGGCCG	TGAKTTGGAT
24401	CCCCCCAAGG	PCATGAGGAC	AGTCATGAGT	GARCTCATCC	Trichicense	טנאמנננננע	CACAGGGGATG	CAAATTTGCA	AGANCANACA	CAGGAGGG"
	GGGGGGTTCC	AGTACTCGTG	TCAGTACTCA	CTCGACTACC	ACKINGGENEG	CATHOGRAPHIC	CTCTCCCTAC	GTTTAAACGT	<b>1CTIGITIES</b>	CTCCTCCCC(%)
24501	TACCCGCAGT	TOCCCACGAG	CAGCTAGGG	GCTCCCTTCA	AACGCCCCCACAG	CCTCCCCACT	TGGAGGAGCG	ACCCAAACTA	ATGATOCCCG	CAGTY:CTCTT
	ATGGGCGTCA	Acceptectic	GTCGATCCCC	CCACCGAAGT	TYXXXXXX	GGACGGCTGA	ACCTCCTCCC	TOCGTITICAL	TACTACCOOC	GTCACGAGCA
		S TABLE	ofte moster							
24601	TACCGTGGAG		CTTCAGTGCA TGCAGCGGTT	CTTTGCTCAC	CCGGAGATOC	AGCCCAAGCT	ACACHANACA	TTGCACTACA	CCITITOGACA	COCCTACGTA
	ATOOCACCTC	GAACTCACGF	ACGTCGCCAA	GAMCGACTG	GGCCTCTACG	TCGCGTTCGA	rerectivist	AACGTGATGE	CCANACCICT	CCCGATGCAT
		Bell								
24701	COCCAGGCCT	GCAAGATCTC	CAACCTGGAG	CTCTGCAACC	TRESTUTECTA	CCTTGGAATT	TTGCACGAAA	ACCOCCTTOO	GCAMACGTG	CFTCAFFCCA
	GCGGTCCGGA	COFFICTAGAG	OFFICEACCITC	GAGACOTTOG	ACCAGAGGAT	GCAACCTTAA	<b>AACGIGCITY</b>	TOCCOGNACC	COTTITICCAC	CAACTAAGOT
		Asch	*							
24801	CGCTCAAGGG	CGAGGCGCGC	COCCACTACO	TCCCCGACTC	CCTTTACTTA	TFICTATGET	ACACCTIGGCA	GACGGCCATG	GGCGFTTGGC	AGCAGTGCTT
	GCGAGTTCCC	GCTCCGCGCG	<b>GCGCTGATGC</b>	AGGCGCTGAC	GCMATGAAT	AAAGATACGA	TETCHACCGT	CTOCCOGTAC	CCGCAAACCG	TOGICACGAA
			Pstl							
24901	DCAGGAGTGC	AACCTICAAGG	AGCTGCAGAA	ACTOCITANAG	CNAMICTEDA	AGNACCTATG	GACCICCTTC	AACGAGCGCF	CCCTGGCCCC	OCACCTOSCY:
	CCTCCTCACO	TTCCAGTTCC	PCGACOTCTT	TCACCATTTC	GTTTTGAACT	TCCTCGATAC	CTOUCHGANG	TTGCTCGCGA	GOCACCOOCG	CGTGGACCGC:
25001	GACATCATT	TECECOANCE	CCTGCTTANA	ACCUTACAAC	ARKSTOTOCC	AGACTTCACC	ACTICANAGEA	TGTTGCAGAA	CTTTAGGAAC	THEN TOCTA
	CTOTAGTABA	AGGGCTTGC	GGACCEANTIFF	TRACARCETTG	TCCCAGACGG	TCTGAAGTGG	TCAGTTTCGT	ACAACGICIT	GANATECTTG	AAATAGGATI'
25101	AGCGCTCAGG	AATCTTGCCC	GCCACCTGCT	GTGCACTTCC	TAGCCIACTIT	GISCCCATTA	ACTACCCCCA	ATGCCCTCCG	CCCCTTTGGG	GCCACTRECTA
	TOGGGAGTCC	TTAGAACGGG	COGTOCACCA	CACGTGAAGG	ATCCCTGAMA	CACGGGTAAT	TCATGGCGCT	TACOGGAGGC	GCCGARACCC	COGTGACGAT
	Pati	,								
25201	CCFFCFGCNG	CTAGCCAACT	ACCITIOCCITA	CCACTICTIGAC	ATAATCCAAG	ACCHINACCEG	TICACOSTICTA	CTRIGAGRETIC	ACTOTOGOTO	CAACCTATES
	CCAAGACGTC	GATCGGTTGA	TOGAACCCAT	GGTGAGACTG	TATTACCITC	TOCACTCRCC ACTOCCAGAT	ACTOCCAGAT	GACCITCACAG	TCACAGCGAC	GITGTATALT
						\$	101 	Psti		
25301	ACCCCGCACC	<b>GCTCCCTGGT</b>	TIGGAATICG	CACCTCCTTA	ACCIANAGICA	AATTATCGGT	AAITTATCGTT ACCTITIGAGC	TECAGGGTCC	CTCGCCTGAC	GANNAGTECT
•	TOCCOCOTOC	CGAGGGACCA	AACGITAAGC	GTCGACGAAT	TOCTTTCAGT	TTAATAGCCA	TOGARACTOG	ACGTCCCAGG	CACCCCACTG	CITTICAGO.
25401	COGCICCOOC	GITGAMACTC	ACTCCGGGGC	TOTOGACGTC	<b>GGCTTACCTT</b>	CCCAAATTTE	TACCTIGAGGA	CTACCACGCC	CACGAGATTA	GOTTCTACGA
	CCCCAGGCCC	CAACTITIGAG	TEMOSCECCE	ACACCTGCAG	CCGAATGGAA	CCSTTTAMC	ATCCACTCCT	CATECTOCO	GTCCTCTAAT	CCAAGATUTT
25501	AGACCAATCC	COCCCOCCTA	ATGCGGAGCT	TACCRECTASE	CHIATTACCC	NATICICACAT	TCTTROCCAA	TTGCAAGCCA	TCAACAAAGC	CCCCCANGN
	TCTOCTTACG	GCCCCCCCAT	TACCCCTCGA	ATGGCGGALIS	CANTAATINGG	Trecentition	ACAACCOGIT	AACGTTCGGF	AGITICITETICG	GECKETTICH:
25601	TTTCTGCTAC	GANAGOGACG	-	THOCACCCC	AGTCCGGCCA	OCACCTCAAC	CCAATCCCCC	COCCOCCOCA	GCCCTATCAG	CAPCARECTS.
	AAACACCATG		CCCCAAATG	AACCTREGGE	TCACCCCCT	CCTCGACTTG	CONTRACTOR	COCCOCCO	COCCATAGIC	GICGICGO

Figure 15P

#### DMRKACISgag MER682

				Pstl						
25701	GGCCCTTGC	TACCCAGGAT	PRECENCIANT OCCACETANA	AAGAMOCTIC ALK TITALY TOLD	ALK THE VERTICE	ישבייניביים			CAGTCAGGCA	CACCACACTET
•	CCCGGGNACG	AAGGGTCCTA	AAGGGTCCTA CCGTGTGTTT TTCTTCGACG TCCACCGG	TTCTTCGACG	TYTE STATE OF THE	۲		TTATGACCCT	greacteest	CHICCHCCAN .
						tore				
25801	TOCACCACTA	CCACCACCAC	ATGATCAMG	אביונאפטיפאפ נזידאהאהיהאם		CANGCITTCCG	ACCITCCAAGA	GETCTCACAC	GAAACACCGT	CACCETOCK .
	ACCTGCTCCT		TACTACCTTC	TGACCCTUTC	CGATCTCCTC:	CTTCGAAGGC	TCCAGCTTCT	CCACAGICTG	CTTTGTGGCA	GTCCCCAGC('A
25901	CGCATTCCCC	TCGCCGGCGC	CCCASAAATC	GGCAACCGGT	TECAGEATEG	CTACAACCTC	CGCTCCTCAG	0000000000	CALTGCCCGT	TOTALCEACCC
	GCCTAAGGGG	AGCGGCCGCG	CONTENTAG	CCGTTTGGCCA	AGCITCGTACC	GATESTEGRAG	CCGAGGAGTC	ניבססכסכיב	GTGACGGGCA	ACCOCATECTO
26001	AACCGTAGAT	GGGACACCAC	TOGANCCAGG	GCCCGTAAGT	CCAMICAGCC	CCCCCCTTA	GCCCANGAGC		CCAAGGCTAC	COCHCATOO!
	TYGOCATCTA	ceercreers	ACCTIGGICC	COCCATTCA	<b>GSTTCGTCGG</b>	CRECHICANT	COCCINCICO	TIGHTGICGC	GGTTCCGATG	OCGNGTACC(;
26101	<b>OCCOOCACAA</b>	GAACGCCATA	GTTCCTTGCT	TOCANGACTO	TECHCICCAAC		CCCIRCUST		CATCACGGCG	المحتالات المسائدات
	COCCORDIT	CITICCOCTAT	CAACCAACGA	ACCITICAGAC	ACCCCCGTTG	TAGAGGAAGE	GCCCCCCCAA		GTAGTGCCGC	ACCHITAACCO!
26201	CCGTAACATC	CTGCATTACT	ACCORCATET	CTACAGCCCA	TACTRICARCES	GCCXXCVGCCG	CAGGNACAGC	ACCCCCCACA	CAGAAGCANA	מאכנועכבפניע
	OCCAPITCTAG	GACGTANTGA	TGCCAGTAGA	GATGTCGGGT	ATGACGTGGC	COCCOICCCC	Greeneres	reaccount	orcricorn.	CCGCTGGCCT
26301	TAGCAAGACT	CTGACAAAOC	CCAAGAAATC	CACAGCGGCG	GCARCAGCAG	CACCACACACC	GCTGCGTCTG	GCGCCCAACG	ACCCUTATO	CACCCGCGAG
	ATCOTTCTGA	GACTOTTTCG	GGTTCTTTAG	GIGICOCCOC	conconconc	CICCICCICG	CGACGCAGAC	CGCGGGTTGC	TTGGGCATAG	CTGGGCGCTC
26401	CTTAGAAACA	GUATTETECC	CACTCTGTAF	CCTATATTIC	AACAGAGCAG	GROCCANGAA	CANGACCTCA	AAATAAAAA	CADGICTICTO	CCATCCCTCA
	GAATCTTTGT	CCTANAAAGG	GTGAGACATA	CCATATAMG	THENCIONIC	CCCGGTICTT	GTTCTCGACT	TITATITIT	GICCAGAGAC	CCTACCCACT
26501	CCCGCAGCTG	CCTOTATCAC	ANAGCGNAG	ATCARCTICG	GCGCACACTG		AGGETETET	CACTAMATAC	TOCOCCACTOR	
	GOOCGTCGAC	GCACATAGTO	trincectine	TAGTCCAAGC	CCCCTCCCAC	כדוכוסכפככ	TCCGAGAGAA	GICATITATO	ACCCCCCACT	
26601	CTAOTITICOC	OCCUPIENCE	ANTITAACC	CCCMANCTA	CGTCATCTCC	AGCGGCCACA	CCCGGCGCCA	GCACCTGTTG	TCAGCGCCAT	TATTARGCAAG
	GATCAAAGCG	COCCANAGAG	TITARATICG	CGCTTTTGAT	CCACTAGAGG	TCCCCCCTCT	GOCCOCCOCOT.	CCTCGACAAC	AGTCGCGGTA	ATACTICGITIC
26701	GALATTECECA	COCCCTACAT	OTCGAGITIAC	CACCCACAM	TOCCACTTOC	<b>GOCTOGAGCT</b>	CCCCAAGACT	ACTCAACCCG	AATAAACTAC	ATGACCCCG
	CITTAAGGGT	OCOGOATGTA	CACCTCAATG	Greenent		ACCCTGAACG CCGACCTCGA	COCCUTICA	TCACTTCCCC	Trattigato	TACTCGCGCC
		EcoRy			n 3	Econ1				
26801	GACCCCACAT	GATATCCCOG	GTCAACGGAA	TACOCOCCCA		CCCANACCGA ATTICTICATION		TATTACCACC	ACACCTCOTA	
	CTOCCOTOTA	CTATAGGGGCC		ATGCGCGGGT	CROCHITICOCT	TAAGAGGACC	THETECOCCE	ATANTOGTOG	TOTOGAGCAT	-
26901	Tececorage	TOCCCOCTG	CCCTGGTGTA	CCAGGAAAGT	CCCCCCCCCCA		ACTITCCCAGA	GACGCCCAGO	CCGAAGTTCA	
	AGGGGCATCA	ACCOGGGGAC	GGGACCACAT	GGTCCTTTCA	CACCCACCACT	GUTTACACACCA	TGAAGGGTCT	CHECOGOSTICE	GGCTTCAAGT	CTACTGATTG
27001	TCAGGGGCGC	AGCTTGCGGG	COCCUTICGE	CACAGOGING	<b>CONTRACTOR</b>	GCAGGGTATA	ACTICACCTGA	CANTCAGAGG	GCCAGGTATT	
	AGTECECCOCO	TEGNACOCCC	GCCGNANGCA	OTOTOCCACG	כבאטכטפנאכ	CCTCCCATAT	TCAGTGGACT	GITAGICICC	CGCTCCATAA	
27101	ACGAGTCOOP	-			GACATTICAG	ATCGGCGGC	CCCACCACTC	PICATTICACE	CCTCCTCAGG	CANTECTAAC
	TECTICACCCA	CTCGAGGAGG	GARCCAGAGG	ראפורוופרו			occupand.			
27201	ALAKSUNG BULL	والمنطاب المسادة	AGCGCGCTC	TRICAGOCATT	CCAACTCTCC	ANTITATICA	CHACTTICTO	CCATCOGICT		ACTITAACCC CITICICOGGA
	AGACGTCTOG		TCCCCCCGAG			TTAAATAACT	CCTCANACAC	GGTAGCCAGA	TGAAATTOGG	GAAGAGCCCT

Figure 1502

1017	CTREEGOCE	ACTATCCOGA 1	TCAATMTATT	CCTAACTTIG	ACCITICATION O	ביניער ביניער ביניער כיני	GACGGCTACG 1	-	NAGTOCAGAG	GCAGAGCAAC
, ,	CENTROCCEGO	_		GGATTGAAAC	TRECHERCIATER (	כבוניאטכניני נ	כונטניניניאדה כ	TYPETTACA	TTCACCTCTC	CONCINCIANT
27401	TUCGCCTGAA	ACACCTGOTC	CACTGTCCCC	CCCACAACTR					CCCGAGGATC	ATATCCAGGG
	ACCCCCCACTT	TGTGGACCAG (	GTGACAGCGG	COCTOTICAC	מאארימממכ	CTGAGGCCAC 1	TCAMACGAT	GNAACTTANC	OGGCTCCTAG	TATAGCICT
27501	CCCGGCGCAC	OCCUPECTOC	TTACCGCCCA	COGNGARCTT					TAGTTOAGCO	GGACAGGG :A
	GOGCCGCGTG	CCCCAGGCCG	AATGGCGGGT	CCCTCTCGAA	CCCCCATCGG	ACTAAGCCCT (	CAAATGGGTC	GCGGGGGACG	ATCAACTUGG	. consucco
3					CATHERCEATICE	******	TOCCATCICE	Greensagta	TAATAAATAC	AGNANTTA: A
27601	CCCTGTGTTC	TCACTGIGAT	TIGLAMCTOT	GCATTGGGAC					ATTATTATO	TCTTTAAT!
11101	SCONCACTORS OF STREET	NOT CHECKET	CCATICATA	AACGCCACCG	TETTEACCCG		CCAAGGGGAA	CCTTACCTOO	TACTITITANC	ATCTCTCO .
3	TATATOACCC	CGAGGATAGC	CGTACCACAT	THECOGNOC	AGANGTGGGC		CONTCOCUT	CCAATCCCACC	ATGAMATTO	TAGAGAGGG:A
27801	CHARLATTERA		AACCCAGACG	GAGTGAGTCT	ACCIACIAGAAC	Chereconde	TCACCTACTC	CATCAGAAAA	AACACCACCC	TCCTTACCT:
	GACACTAGAT		TROCTCTGC	CTCACTCAGA	TGCTCTTG	GAGAGGCTCG	ACTCGATCAG	GRAGICTITI	rrcrocroco	ACCUATIOGAC
27901	CCGGGAACOT	r ACCAGNOCOF	CACCOCCOC	TOCACCACAC	CTACCGCCTG		AGACTTTTTC	COCACAGACC	TCAATAACTC	TGTTTACCAG
	GOCCETTOCA	A TOCTCACOCA	GINGOCCOGCG	ACCITOCITOTO	CATCCCCCAC	TCCCATTIOG	TCTGAAAAA	<b>GCCTGTCTGG</b>	AGITATTOAG	ALMANIGE IC
28001	NACAGGAGGT	P GAGCITAGAA	AACCCTTAGG	GTATTAGGCC	ANARGOGGAG	CTACTGTGTG	GITTATCAAC	ANTICAROCA	ACTUTACOOG	CTATTCTAAT
	THETECHECA	A CTCGAATCTT	TTGGGAATCC	CATAATCCGG	Trrececenc	CATCACACCC	CAAATACTTG	Transferede	TCACATCCCC	GATARCAT"
	•	Xbal								
28101	TCAGGITTCI	T CTAGAATOOD	<b>ACTROGESTY</b>	ATTETETET	TREFGRENCE		ATACTARCOC		AAGGCTCGCC	OCCTOCTE: 1
	AGTCCAAAGA	A GATCITAGCC	<b>ECAACCCCAA</b>	TAAGAGACAG	AACACTANGA	GANTARGAA	TATCATTOCG		TTCCCAGCGG	CCCACGACAC
28201	TOCACATTING	3 CATTRATTOT	CAGCTTTTTA	AACGCTTGGGG	TCGCCACCCA	MGATGATTAG	GTACATAATC			GTCAGCCCAC
	ACCTICTAAAC		GTCGAAAAAT	TTGCGACCCC	AGCGGTGGGT	TCTACTAATC	CATGTATTAG	GATCCANATO	AGTOGGAACO	CAGICGASIVI
	Kord									. 100454554
28301	COTACCACC	C AMAGGTGGA	TTTTANGGNG			CCCACCTGAA	GCTWATCHGT			
	CCATGGTGGG		AAAATTCCTC	GGTCCCACAT	TACAATKITAA	GCCTCGACTT	CGATTACTCA			
28401	ATCAAAAGCT	r ocmatrosc	CACAAAAACA	AAATTOCCAA	GTANKETETT	TATECTATT	MICARCCARG		CACTATAATO	
	TACTITICGA				CATACGACAA	ATACCATAAA	ccercoence	ACTOTOANGE	CTCATATTAC	AATOTCAAAA
			•	RS11071						
28501	CCACACTAAA	A ACTICATABAA	CHTTATCTA	TACTITITICCA	TITITATICALA	TOTACGACAT	TACCATGTAC	ATCAGEAAAC	-	
	CONCEANT			ATGAMMGGT	ANNTACTIT	ACACCCTGTA	ATCGTACATG	TACTCGITIG	TCATATTCAA	_
28601	CAAAATHGEG			TOCTOCACTO	CTATCCTAAT	TACAGTGCTC	<b>actititiscict</b>	GTACCCTACT	CTATATTAAA	TACANARGCA
	GITTITAACAC		ACCGTGAAAG	ACGACGTGAC	CATACGATTA	ATCTCACCAC	CCAAACCAGA	CATGGGATGA	GATATAATTT	ATCILITY
28701	GACGCAGCTT	T TATTGAGGAA	AAGAAAATGC	CTTAATTTAC	TANGITACAA	-	-	_	_	AACMANTT
	CTGCGTCGAA	-		GAATTAAATG	ATTCAATGTT	TCGATTACAG		-	_	
28801	AAAAGITAGC	C ATTATAATTA	_			-			achard and	TATATICERCY
	TITICAATCO	G TAATATTAAT	CTTATCCTAA	ATTTGGGGG	CCAGTANAGG	ACCAGITATE	GTAAGGGGAC	DIDWITCH.		

28901	GCGCTACAAC	CTTGAAGTCA	COCTROCTOR	ATCHURCAT	CHIACTTRIGG	CCAGCACCTG	TCCCGCCGAT			בניאנבכאניינ
	-	GAACTTCAGT	CCEMICRANC	TACAGITECTA	GACTERANCC	COLCORATAC	AGGCGCCTA	ANCAAGGTCA		בור אבאבאר א זכ
29001	TAACAGAGAT	GACCAACACA	ACC MACHOCAG TOST TRACECC	CCGCCGCTAC	CCCACTTACA	TUTACCACAA AGATEXTICAT	ATACACCCCA	ACTITICTOCC TCANGACG	AAACAGTTAT	ACTOGGATAA TGACCCTATT
29101		recreamen	CCATAGCGCT	TARSTITION	TKHTCTTATTA			CTARAGEGEA	AACGCGCCCG	ACCACCCATC
	GAACCCOTAC	ACCACCANGA	<b>GCTATCGCCA</b>	ATACAAACAT	ACGGANTANT	NATACNOTON		GATTICGCGT	TTGCGCGCGC	TOG IT I STITLE
29201	TATAGICCCA	TCATTGREET	ACACCCAAAC	MITCATISTA	TCCATACATT	GENCACAPTIG	AAACACATGE	TCTTTICTCT	TACACTATCA	TTANATGAGA
		AGTAACACGA	TOTOGGITTE	TTACTACCTT	AGGTATCTAA	CCTGCCTCIAC	TITICICITACA	ACANANGAGA	ATCTCATACT	AATITACTCT
	R	Xhol								
29301		CCAGTTTTTA	TATTACTGAC	CCTTK4TTGCG	CTITITION	CTITITIONS CONSCINCAN		GTTTCTCACA		CTCCATTC A
	GTACTAAGGA	GCTCAAAAT		ATANTGACTG GGAACAACGC	GNANNANCAC	GNAMANCAC OCACCARRETTS	TANCCGACGC	CAAAGAGTGT	AGCTTCATCT	GACCTAAG
					Ps	Pstl				
29401	GCCTTCACAG	TCTATTTGCT	TTACCGATTT	<b>GICACCCTCA</b>	CACTENTERS	CACTENTETE CAGCETEATE	ACTIGITICA	TCCCCTTTAT		GACTOGGTCT
	COGARGTOTC	ACATANACCA	AATOCCTANA	CAGTGGGAGT		GCGAGTAGAC GTCYAGAGTAG	TCACACCAGT AGCGGAATA	AGCGGAAATA	CCTCACCTRA	CTGACCCAGA
							Ecof			
29501	Create Court	TOCATATOR	AGACACCATC	CCCAGINCAG	GGACAGGACT	ATAGCTGAGC	TTCTTAGAAT TCTTTAATTA	TCTTTAATTA	TOMATITAC	TOTOACTIT!
	CACACGCGAA	ACCTATAGAG			CCTGTCCTGA	TATEGACTEG	ANGNATICITIA AGNAATTAAT	AGMATTAAT	ACTITABATO	ACACTGAAAA
29601	CTGCTGATTA	THECACCCT	ATCTGCGTTT	TGTTCCCCGA	CCTCCAAGCC	TCAAAGACAT	ATATCATGCA	GATTICACTICG	TATATCCAAT	ATTCCAACT T
	GACGACTAAT	AAACGTGGGA	TAGACGCANA	ACANGOGGCT	CCACCTTCCC	AGTITICAGEA	TATAGTACGT	CTAAGTGAGC	ATATACCITIA	TAAGGTTCAA
							Pstl			
29701	CCTACAATGA	AAAAAGCGAT	CTTTCCGAAG	CCTCCTTATA	TOCARTCATO	TCTGTTATGG	TOTTICTOCAG		GCCCTAGCTA	TATATCCCIA
	CGATGITACT	TTTTTCCCTA			ACCITAGTAG	AGACAATACC	ACAAGACGTC	ATGGTAGAAT	CGGGATCGAT	ATATACKGAT
29801	CONTRACATE	COCTOCAACO	CAATAGATGC	CATGMACCAC	CCANCITICC	כבפכנובבבנונוב	TATOCTTCCA	CTCCAACAAG	TTOTTGCCCG	COCCITICAL
	CCAACTGTAA	CCGACCTTGC				COCCCCCCCCC	ATACGAAGGT	GACGITOTIC	AACAACGGCC GCCGAAACA :	GCCCANNEY :
		,							2 2	ill mare
									Pott	
29901	CCAGCCAATC	Acceptace	ACCTICACC	ACCUCCACTO	AANTCACCTA	CTTTAATCTA		ACAGGAGAG ATGACTGACA	CCCTAGATOT	-
	CONCECTING				TITAGICGAL	GANATTAGAT	TOTOCTOCTO	TACTGACTOT	GGGATCTAGA	TCTTTACCTG
30001	GCAATTATTA	CAGAGCAGCO	CCTCCTAGAA	AGACGCAGGG	CAGCGGCCCA					TIGCACCAGE
	CCITIAATAAT	GICTCGTCGC	: GCACCATCTT	rendedance:	CHCGCCGGCT	cerrenced	TACTTAGITIC	TCCACCTTCT	GTACCAATTG	AACCHGGTCA
10101	GCAAAAGGGG	TATCTTTTGT	CTCGTANAGG	NOCCCANGE	CACCTACGAC	AGTANTACCA	CCGCACACCG			CCAAGCCT**
	CONTINCOCC	ATAGANAACA	GAGCATTICG	recentres.	GTOGATICCTO	TCATTATION	GGCCTGTGGC			_
30201	GAANTTOOTO	GECATOGEG	CACANAAGCC	_						
	CITIBACCAC	CAGTACCACC	: CRCTTTTCCG	GTAN	TCACTCCTCA	GCCATCTTTG	GCTTCCGACG	TAGTGAGTG	GAACAGTICC	וואארורוא
					3				CAPP CHAPABA	PH-BGHTBG
30301	CACACCCC		TTATTANGAC CCTGTGCGGT AATAATTCTG GGACACGCCA	GAGTITICTAS	GAGTITICTAS AATAAGGSAA	ATTCATTATT		ATTICCTACT		TACTCAATCG

## pMRKAd5gag MER682

30401	AAATTTCTGT	CCAGTITATI	CAGCAGCACC	rectroxect	CCTCCCAGGT	CTEATATING	AGCTTCCTCC	TOCHOCAAA	CHITCICCAC	ANTCTABATG
30501	CHACACTCA		TCCTGTCCAT	CCGCACCAC	TATCTTCATG	THEFTER AGA	TCAAGCGCGC	AACACCCTCT	CAACATACCT	TCAACCCCC:T
30601	GTATCCATAT		CCCGTCCTCC	AACTSTRECCT TTGACACGGA	THE TYPETE AMGNATEND	CTCCCTTTGT	ATCCCCAAT	CCCAAAGITC	AGAGTCCCCC	TCGGGTAC1 :
30701	TCTTTGCGCC	TATCCGAACC	TCTAGITACC	TCCAATCOCA	TKICTTISCOCT	CAAAATCGGC	AACTO CTCP	CTCTGGACGA	GCCGCAAC	CTTACCTCCT:
30801	AAAATGTAAC		CCACCTCTCA	AANAAACCAA	GTCAAACATA	AACCTGGAAA	TATCTCCACC	CCTCACAGTT	ACCTCAGAAG	CCCTAACTOT
30901	GGCTGCCGCC	-	TOGICOCOO	CAACACACTC	ACCATGCAAT	CACAGGCCCC	GCTAACCGTG	CACGACTCCA	ACTINGCAT	TOCCACCCAA
31001	OGACCCCTCA CCTGGGGAGT		AGGAAAGCTA	GCCCTGCAAA	CATCAGGCCC	CCTCACCACC	ACCCATAGCA	GTACCCTTAC	TATCACTOCC	TCACCCCTT. AGTGGGGGA
31101	TAACTACTOC	CACTGGTAGE	THOCOCATHO	ACTTICAANGA	COCCATTIAL	ACACAAAATG	GAAAACTAGG	ACTAMAGIAC TGATTICATO	CCCCAAGGA	TOCATOTAL .
31201	AGAGGACETA	AACACTITIGA	CCGTAGCAAC	TOGTCCAGGT	CACTGATAAT	ATAATACTTC	CTTGCAAACT	AAAGITACTO	GAGCCTTGGG	TITIGATICA MANCIANI
31301	CAAGGCAATA	TGCAACTTAA ACGTTGAATT	TGTAGCAGGA	GCACTAAGGA	TRGATTCTCA	MACAGACGC	CTTATACTTG	ATCITACTEA	ACCOPATICAT.	CCTCAAAACC CCACTTTTT
31401	ACTANTET TTGATTTAGA		CAGGGCCTC	TTTTTATAAA AAAAATATTT	CYCAGCCCAC	AACTTCCATA	TTAACTACAA AATTGATGTT	CAAAGOCCTT	TACTFOTTTA	CACCTICAN
31501	CAATTCCAAA	MAGCITICAGO	TTAACCTAAG AATTCGATTC	CACTGCCAAG	CCCANCTACA	TTCACCCTAC	AGCCATAGCC TCGGTATCGG	ATTAATGCAG	GAGATGGGCT	TORATTICO: P ACTTANACCA
31601	TCACCTAATG AGTGGATTAC	CACCAAACAC	AAATCCCCTC	AAAACAAAA	THREECATED	CCTAGAATTT	GATTCAAACA	AGGCTATGGT	TCCTAAACTA	CCTRACTCCC
31701	1TAGTTTTGA AATCAAAACT	CACCACAGGT	CCCATTACAG	TAGGAAACAA	ANATANTCAT	AAGCTAACTT	TUTOCHCCAC ACACCTRAFTG	ACCAGCTCCA TOGTCGAGGT	TCTCCTAACT AGAGGATTGA	<b>GTAGACTAAA</b> CATCTGATTT
31801	TOCAGAGAAA ACOTCTCTTT		TCACTTTOOF AGTGAANCCA	CTTAACAAAA	TCTCX CACTC	MANTACTITICS	TACAGITITCA	CAAAACCGAC	TTAAAGGCAG	TTTGGCTCCA
31901	ATATCHERA TATAGACCTT	7	TGCTCATCTT	ATTATAGAT	TTCACGAAAA		CTAMCAATT CATTTGTTAA		CCCAGAATAT	TL. JANCTITA ACCTITANAT
32001	GAAATGGAGA CTTTACCTCT	GAANTGARA TETTACTGAA CITTACCICT AGAATGACTT	OCCACACCCT CCGTGTCCGA	ATACAAACGC TATGTFTKECG	TGTTGGATTT	ATGCCTAACC TACGGATTGG	TATCAGCTTA	TCCAAAATCT	CACCOTANAA	CTCCCAAAAG

Figure 15T

# PMRKAd5gag MER682

32101	TAACATHOTC	AGICAAGITIT	ACTTANACCS TGAATTINGC	ACACANANCT	AAACCTTGTAA	CACTAACCAT	TACACTARAC	GCTACACAGG CCATGTGTCC	AAACAGGAGA	CACAACTC!.A GTGTTRAG: 7'
32201	AGTOCATACT TCACGTATGA	CTATOTCATT	Treateceae Angtaceets	Transpord Arcagaeges	ACAM TACAT TOTTCATCTA	TATTACTTATA	TTTGCCACAT NACCGCTCTA	CCTCTTACAC	TTTTTCATAC	ATTRICCCAN:
32301	AATAAAGAAT	CCAMCACAA	ATCITTEMAC	CACAMATAN	THEANTHEEN	GAAAATTTICA CTTITIAAAGT	ACACTETET TENCH	CATTCAGTAG	TATAGCCCCA	CCACCACATA
32401	GCTTATACAG CGAATATGTC	ATCACCGTAC	CTTANTCAM	CTCACACAAC	CCTAGENTIC	AACCTERCOC	CTCCCTCCCA	ACACACAGAG	TACACAGTCC	TTR: TECCI V:
32501	GCTGGCCTTA	AAAAGCATCA	TATCATCCCA	AACAGACATA	TTCTTMSGTG	TTATATTCCA ANTATANGGT	CACAGITTICE	TOTCGAGGCA	AACGCTCATC TTGCGAGTAG	AGTONTATI . TCACTATA
32601	ATAAACTCCC TATTTGAGGG	COOCCAGCTC	ACTTAACTTC TGAATTCAAG	ATCHCCCTGT TACACCGACA	CETACHTGCTG	ARCCACAGGC TECTGTCGAA TUGITGTCGG ACGACAGGTT	TOCTGTCCAA ACGACAGGTT	CTTGCCGTTG	CTTAACGGGC GAATTGCCCG	CCUCITICCIA
32701	AAGTECAGGE	CTACATOGGG	GTAGAGTCAT	AATCCTCCAT	CAGGATAGGG GTCCTATCCC	PSI CONTRODUCT GCAGCAGCGC GCCACCACGA COTCGTCGCGC	GCAGCAGCGC CGTCGTCGCG	GCGAATAAAC	TGCTGCCGCC ACGACGCGG	OCCUCACION CONTRACTOR
32801	Path CCTGCAGGAA GGACGTCCTT	TACAACATGG	CAGTOGICTC		ATTCGCACCG	CCCGCAGCAT	AAGGCGCCTT TTCCGCCCAA	GTCCTCGGG	CACAGCAGGG	CACCCITGA!
32901	TCACTTAAAT	CAGCACAGTA	ACTOCAGCAC TCACCTCGTG	AGCACCACAA	TATTCTTCA ATACAGTT	AATCCCACAG TTAGGGTGTC	TGCAAGGCGC	TOTATCCAAA	GCTCATGGGG	GOGACCACAG
33001	AACCCACOTO TTCCOTGCAC	GCCATCATAC	CACAAGCGCA	GCTAGATTAA CCATCTAATT	GTAGGGACCC CACCGCTGGG	CTCATAAACA	CCCTCGACAT	ANACATTACC TTTGTAATGG	TCTTTTGGCA AGNAAACCGT	TCTTCTAATT
33101	CACCACCTCC GTGGTGGAGG	COCTACCATA GCCATOCTAT	TAAACCTCTG	TABACCECTO ATTABACATO GCGCCATICCA ATTTGCACAC TAATTTGTAC CGCGSTAGGT	GCGCCATCCA	CCACCATCCT	NACCAGCTO	GCCAAAACCT GCCGGCCGGC CGGTTTTGGA CGGGCGGCCG Ecofly	000000000	TATACACTUA: ATATUTGAC:
33201	AGGGAACCGG TCCCTTGGCC	GACTGGAACA	ATGACAGITEG	AGAGCCCAGG TCTCGGGTCC	ACTECTTAACE TGAGEATTGS	ATCCTAGTAG TACCTAGTAG	ATCCTCGTCA TACGAGCAST	TGATATCAAT ACTATAGITA	GTTGGCACAA	CACAGGCACA GTGTCCGTGT PSI
33301	CCTCCATACA	CTTCCTCAGG	ATTACAAGCT TAATGTTCGA	CCTCCCGCGF	TAGAACCATA ATCTTGGTAT	TCCCAGGGAA	CAACCCATTC	CTGAATCAGC	GTAAATCCCA CATTIAGGGT	CACTTSCAGGG GTGACCTCT:
33401	ANGACCTOCC TTCTOGAGCG	ACCITACTCA	CGTTCTGCAT	TGTCANAGTG ACAGTTTCAC	TTACATTCCG	CACACCACCATA COTTCOTTCOTA	ATGATCCTCC TACTAGRIAGG	AGTATOGTAG TCATACCATC	CCCCCCAAG	TGTCTCANA ACAGAGTTT F
33501	CCTCCATCTO	GATCCCTACT	CATACCCACAGE	CCCCCCCCTCTGT	ACCCACATEG TOCCTCTAGE	NOTINGENIEST ACAACCAGCA	AGTGTCATGC TCACAGTACG	CANATGGNAC	OCCOGACOTA CGGCCTGCAT	GICATATATA:

Paye

						Representative and the Resident Residen		March & College	CTRACTBATA .	CACHCHETEA
1096	CTGNAGCANA	ACCARGINGED WATHER ACTOR	COCCINIACAN ACMINATURAC GIRCIAC CONCINCIONAL CONCINCIONAL TRANSPORTER C	ACMINACTIC C						CTCAGAGAET"
11701	AAGCATCCAG	Granice	GCTTCGGGTT	CTATGTANAC '			TEATAACATC	CACCACCGCA	GANTAAGCCA	CACCCAGGG
	Tregradue	COCCOCICAC		GATACATTIC AGGAAGTACG			ACTATTOTAG	GTCGTGGCGT	CTTATTCGGT (	Greencear
13801	ACCTACACAT		ACTEACHERE	המפשההתה ההאחתה החאהאת באד	ההאהאהכדה		CHARTERIA	TTATTCCAAA	AGATTATCCA	AVACCTI WAA
	TCCATCTCTA		TCAGTGTGTG	כככתככתכנכ	CCTTCTCTAC		CHARAMARA	AATAAGGTTT	TCTANTAGGT	TITCGAGITT
	Bott	9								
13901	ATGAAGATCT	ATGAAGATCT ATTAAGTGAA	COCOCTOCOC	Tecentrace	TYGICANACT	CTACACCCAA	NGNACAGATA	ATGGCATTTG		CACANTEGET
	TACTTCTAGA	TAATTCACTT		AGGCCACCC	ACCAGITICA	GATGTCGGTT	TCTTGTCTAT	TACCGTANAC	ATTCTACAAC	GTGT11ACCC17
34001	TCCAAAAGGC	AMACGGCCCT	CACGECCAAG	TECACETARA	GGCTANACCC	TTCAGOGTGA	ATCTCCTCTA	TAMACATTICC	ACCACCTTCA	ACCATGCCCA
	AGGITTICCO	-	GIGCAGGITC	ACCTGCATTT	CCGAITTICGG	MGTCCCACT	TNGAGGAGAT	ATTTOTAGG	TCOTCOAACT	TOGTACOCCT
10191	AATAATTCTC	ATCTCGCCAC	CITCICALIA	TATCTCTAAG	CAMATCCCGA	ATATTAAGTC	CGGCCATTGT	AAAAATCTGC		CCTCCACCTT
	TTATTAGAG	_	GAAGAGITTAT	ATAGAGATIC	GTTTAGGGCT	TATAATTCAG	CCCCCTAACA	TITTAGACG	AGGTCTCGCO	GCAGCTGCAA
14201	CAGCCTCAAG	CAGCGAATCA	TGATTGCAAA	AATTCAGGTT	CCTCACAGAC	CTCTATAMGA	TTCANAGCO	GAACATTAAC	AAAAATACCO	CGATCCCGTA
	GICGGAGITIC		ACTAACGITY	TTAAGTCCAA	GGAGTGTCTO	GACATATTET	MOTFITCOC	CTTCTAATTG	THITTATOGC	GCTNGCGCAT
34301	COTECCTICG	CAGGGCCAGC	TGAACATAAT	COTTACAGGIC	TGCACCGGACC	ACCCCCCCCA	כיווכככככככ	ACCAACCATG	ACANARGNAC	CCACACTGAT
	CCAGGGAAGC	_	ACTIGIATIA	_	ACGTGCCTGG	ACGIGCCTGG TCGCGCCGGT	GAAGGGGCOG	<b>TCCFTGGTAC</b>	TGTTTTCTTG	GGTGTGACTA
					토	1100				
34401	TATEACAGGC		ATACTCOGAG CTATOCTAAC	CAGCGTAGCC	CCCATCTAAG	CCCATGTANG CTTGTTGCAT	GGCGGCCAT	ATMANATOCA	AGGRECTECT	CAAAAAATCI
	ATACTGTGCG		GATACGATTG	GTCGCATCGG	COCTACATTC	GAACAACGTA	CCCGCCGCTA	TATITIACGE	TCCACGACGA	GTTTTTAG.
34501	COCAMGCCT	COCCCANANA	ACAANGCACA	TCGTAGTCAT	CKTICATGCAG	NTANARGENG	GTAAGCTCCG			ACCATITITIC
	CCGTTTCOGA	A OCOCOTTITI	remicolor	AGCATCAGTA	CGAGTACGTC	TATTTCCGTC	CATTCGAGGC	cricorcoro	TCTTTTTCTO	TCCTANAANG
34601	TCTCAMCAT	מוכיוטכסטטד	THETECATAA	ACACAMATA	ANATAACAM	MAACATTTA	AACATTAGAA			<b>AACAACCC717</b>
	AGAGITITOTA			TCTCTTTTAT	THATHGITT	TITITICINANT	TIGINATCIT	COGACAGAAT	OPTOTOCTTT	THEFTOCOLA
34701	ATANGCATAA	GACCCCACTAC	GCCCATGCCG	CACGITGACCCGT	NAMAAACTG	GICACCOTGA	TTANAMOCA	CCACCCACAG		ATCTCCGGAG
	TATTCGTATT	CTGCCTGATO	CCCCTACGC	CCCACTCCCA	TTTTTTGAC	CAGTCACACT	ANTITITIOGE	GGTGGCTGTC	GACGACCCAG	TACAGACCTC
34801	TCATAATOTA	AGACTCOOTA	AACACATCAG	GITGAITICAC	ATCOSTCAGT	GCTAANNGC	GACCGAAATA			GCAGGGGGTAG
	AGTATTACAT		TIGICIANTE	CAACTAAGTG	TAGCCAGTCA	CCATTITION	CTUGCITTAT	COCCCCCT	TATCTATOGG	CGICCCCCATC
34901	AGACAACATT	P ACAGCCCCCA	TAGGAGGTAT	AACAMATTA	ATAGGAGAGA	ANACACATA	AACACCTICINA	MACCCHCCT		AATAGCACCC
	TCTGTTGTA	_			TATECTETET	THEMOTOTAL	TTCTCCACTT	TTTCCCAGGA	COCATCCCTT	TTATCGTGGG
35001	TCCCCCTCCA		CAGCGCTTCC	ACAGCGGCAG	CCATAACAGE	CAGGETTARGE			MAAAAAACAC	CACTOGACA:
	ACCOCCAGGE	_	GTCGCGAAGG	rereceeste	GGTATTOTCA	CHUCANATOC	TCATTTTTC	TTTTKGATAA	THITTIGE	GROAGCTGIS
35101	COCACCAGCT	r CAATCAGTCA	CAGTGTANA	AAGOGCCAAG	TGCAGAGCGA	GTATATATAG				ACARANARIA
	CCOTOGICGA	A GITTAGTCAGT	GICACATTIT	TICCCGGTTC	ACGRETECTE	CATATATATC	CIKATITITI	ACTOCATTOC	CAATTICAGG	territor
35201	CCCAGAAAAC	COCACOCOAN	CCTACGCCCA	GANACCANAG	CCANNANCC	CACAACTTCC	TEMATICATE		TCCCACGTTA	CONCACTICC
1	GGGTCTTTTC		GGATGCGGGT			GTGTTGAAGG	AGTTTAGCAG		TCAAGCCAAA AGCCTGCAAT GCAGTGAAGG	GCAGTGAAGG

Figure 15V

# PMRKAdSgag MER682

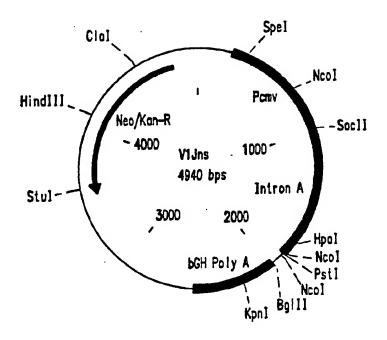
35301	CATTITAAGA GTAAAATICT	AAACTACAAT TTTGATGITA	TCCCAACACA AGGGTTGTGT	TACAAGFTAC TYCOCCCTAA ATGITCAATG AGGCGGGATT	Treascetaa Aggeskaatt	AACCTACGTE ACCCGCCCG TTGGATGCAG TGAGCGGGGC PRd		TTCCCACGCC AAGGCTTCCCG	CCCCCCACG	TCACAAACTC AGTCTTTGAG
15401		THE SECTOR STATES		Constraint	TATATTA	ATKSATICITEAA	FCORI	GGATCTGCGA	CractaAcacette	GATEGEETT .
	GTGGGGGAGT	ANTAGTRIAN	CCGAAGTTAG	GITTIATICC	ATATAATAAC				OCCUTCOGAC	CTACCOGAAG
35501	CCCATTATGA	THEFTETEGE	TTCCGGCGGC	ATCGCGATGC	CCGCGTTGCA				CCATCAGGGA	CAGCTTCAAG
	GGCTAATACT	AAGAAGAGCG	AAGGCCCGCCG	TAGCCCTACG	GGCGCAACGT		-		SCINCIPLE I	CICOMOTIL.
35601	CONCONTIF	CCCCACCTTC	CCATTITIC	CCCCCTTGCT GGCGCAACGA	CCCCAAAAG	CATACACTAC	COOCCCCTGA	GCTCGTAGTG	TITTAGCTO	CCAGTTCAGT
35701	CHECACCOCT	AMCECEACAG	CHICATATARG	NTACCAGGCG TATGGTCCGC	TTTCCCCCTG AAAGGGGGAC	CTTCCAGGGA	CGACGCGAGA	CCTGTTCCGA	CCCTGCCGCT	TACCCCATAC ATGCCCTATY:
35801	CTCTCCOCCT	TTCTCCCTTC	CCCTTCCCAC	GCGCTTTCTC	ATACCTCACG	GACATCCATA	CHCANTICOG	TOTAGGTCGT	TCCCTCCAAO AGCGAGGTTC	CHOCICION.
35901	TGCACGAACC	CCCCGTTCAG			CCCTANCTAT		CCAACCCGGF	AAGACACGAC	TTATCOCCAC	TOOCAGCAGY: ACCGTCGTC
36001	CACTOGTAAC	AGGATTAGCA			CCATCTCACACT	TETTGANGTG AGAACTTEAC	GTGGCCTAAC		CTAGAAGGAC GATCTTCCTG	AGTATTTGGF TCATAAACCA
36101	ATCTOCGCTC	TOCTGAAGCC		CCTTTTTCTC	THESTAGETE	TTGATCCGGC AACTAGGCCG	AAACAAACCA	CCCCTOGTAG	CCCTCCTTTT	TTTGTTTGC. AAACAAACGT
36201	AGCAGCAGAT TCGTCGTCTA	TACGCGCAGA	AAAAAAGGAT	CTCAAGAAGA	TCCTTTGATC	TTTTCTACGG	GGTCTGACGC	TCAGTGGAC AGTCACCTTG	GAMMACTICAC	GTTANGGGAT
36301	<b>ANACCAGTAC</b>	AGATTATCAA	AAAGGATCTT	CACCTAGATC	CTTTTAAATC	ANTCHANGE	ATATATGAGT TATATACTCA	ANACTICATIC	TGACAGTTAC ACTGTCAATG	CANCOLLA
36401	TCAGTGAGGC	ACCTATCTCA TOGATAGAGT		TATTTCGTTC	ATCCATAGET TAGGEATCAA	GCCTGACTCC CCGACTGAGG	CCGTCGTGTA	GATAACTACG	ATACGGGAGG TATGCCCTCC	GCTTACCATY CCAATAG
36501	TOOCCCCAOT	<b>OCTOCANTOA</b> CGACGETTACE	TACCOCCAGA	CCCACGCTCA	CCGCCTCCAG	ATTTATICAGE TAAATAGTEG	AATAAACCAG TTATTTGGTC	CCACCCCCAA	GCGCCGAGCG	CAGNAGTGGT
36601	CCTGCAACTT	TATCCGCCTC ATAGGCGGAG	CATCCACTCT	ATTAATTGTT	CCCCCCTTCG	TAGACTAAGT	AGITCGCCAG TCAAGCGGTC	TTAATAGTTT	GCGCAACGTT	GTTCCCATTG CANCGGTAAC
36701	CTACAGGCAT	COTOCIOTCA	CCCTCCTCGT	TTGGTATGGC	TTCATTCAGC AAGTAAGTCG	TCCGCTTCCC AGGCCAAGGG	AACGATCAAG TTGCTAGTTC	GCGAGTTACA	TGATCCCCCA ACTAGGGGGT	TOTTGTSCAA
			Pwul	11 						
36801	MAAGCGGTT	AGCTCCTTCG TCGAGGAAGC		GICCICCOAT COTTOTCAGA CAGGAGGETA GCAACAGTET	ACTINGTITIS TCATTCAACC	CCGCAGTGTT	ATCACTCATG TAGTICAGTAC	CTTATCCCAG CAATACCOTC	CACTGCATAA	TTCTCTTACT
36901	CACTACCCAT	CCGTAAGATG	CTTTTCTCTG	ACTOGREGAGT	ACTUMCCAA TGAGTTGGTT	CACATACAGA		TOCOCOGACC	CTCAACGAGA	TACCCARCGE

figure 15W

## PMRKAd5gag MER682

ENICACOGA FINITICICO CCACATACA GANCTITAM AGRIACICATE, ATTRAMANC GITCITICOGO GOGAMACIC TCAAGGATCT TACKACAGATAT FITGISCCCT ATTATOCCC GCIGTATCGT CITICAANTIT TYACGACTAG TAACCATTITG CAAGAAGCC COCTITIGAG AGTICCTAGA ATGGGAAAAA BAANECAGT TCAATGTAAC CCACTCGTGC ACTCAACTGA TCTTGAAT CITITAACTT CACCAGGGT TCGGGGAG CAAAAACAG AAGCAAAAT FICTAGGTCA AGCTACATTO GGTGAGGAGG TXXGTTGACT AGAAGTGAAA GAAAATGAAA GHXGGGAA AGACCAACT GTTTTGTCC TACCATTTA	GCCCANAA AGGOATAG GCCACACGS AAATGTRSA TAFICATAGT CTICCTITIT CAATATAT GAAGCATTIA TCAGGATTA TCAGGATTA TCAGGATAGA CCGCAATA ACAGAGTAGA CCGCGATTA TCAGAGATA ACAGAGTAGA CCGCGAATA TCCCCTAATA ACAGAGTAGA ACAGAGTAGA GCGGATAGA ATTAGAATA ATAAACAAA AGGGSTTTCC CCCGAAAAA GCCGAAAAA ATAAACAAAT AGGGSTTTCC CCCGAAAAA GCAACATATTAGAAAA CCATTATAGAAA CCATTATAGAAA CCATTATAGAAA CCATTATAGAAA GCGAAAAAAA CCATTATAGAAAA GCGAAAAAAAAAA	8.
CNACACGODA TANTACCOCO CCACATARCA GAACTITIAAA AGRICITCATE, ATRICAMAIC GITCITICOGO GOGAMACTE TCAAGGATCT TACRRICATAT GITGIOCCCT ATTATOGOCO GOTOTATCOT CITRIAAATHI TR'ACGARTAG TAACCHITTG CARGAAGOCO GOCTITIGAG AGTICCTAGA ATGGCOALAA GAGANCCAGT TCAATGTAAC CEACTCGTG ACTEANGTEA TC'HTRANYAT CITTITACITTT CACCAGGTT TCHGGOGAG CAAAAACAG AAGGCAAAAT CICLAGGTCA AGCTACATTO GOTOGGCACO TRAGITIGACT AGAAGHILITA GAAANTGAAA GINGTGCAA AGCCCACTC GITTITOTCC TROCCTITITA	GCATTTA TCAGAGT CGTAAAT AGTCCCA ACCTGAC GTCTAAG FGGACTG CAGATTC	EDITED BEING
GTYCTYCGGG GCG CAAGAAGCC CGC CACCAKGTY TCY GYYTYCGAA AGAC	CAATATTATT GANG GTTATAATAA CTTV CCCGAAAAAGT GCCJ GGGCTTTTCA CGG	Ecriti Bamti WANTELL GA TI CGAATTET TAAC CT AGGCTTAAGA ATTA
ATTCCAAAAC TAACCTTTG CTTTTACTTG	CTTCCTTTTT GAACTAAAAA CTCACATTTC GCGTGTAAAG	Bambii MAGAATTEGA TYC TYCTYMCCT AGG
AGNACNCANT TYTACGAGTAG TYTHYANATAT AGAAGTUTATA	TACTICATAGE ATTACTATION AGGRETATION TOCOCOANCE	TTTCGTCTTC
GAACTITAAA CTIXAAAITI ACTEAACITEA IXXXIIIGACT	AAATGITISAA TTTACAACTT ATAAACAAAT TATTIGITTA	CACGARGECC
CCACATAGGA GGTCTATCGT CCACTCGTGC GGTCAGCAGG	GGCGACACGG CCGCTGTCGC ATTTAGAAAA TAAATCTTTT	ATACGGTAT TATCCGCATA
TANTACCGCG ATTATGGCGC TCGATGTAAC AGCTACATTG	AGGGAATAAG TCCCTTATTC ATTTGAATGT TAAACTTACA	ACCTATAAAA TGGATATTTT
CAACACCCCA GTTGTGCCCT GAGATCCAGT CTCTAGGTCA	OCCOCANAA CGGCGTTTTT GCGGATACAT CGCCTATGTA	CATGACATTA
37001	37201	37401

Figure 15X



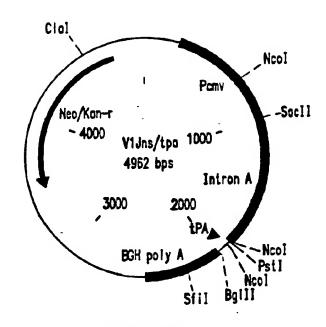


FIGURE 16

GCAGTGGCCCCTGACTGAGGAGAAGATCAAGGCCCCTGGTGGAAATCTGCACTGAGATGGAGAAGGAGGGGCAAAATCTCCA
sGInTrpProLeuThrG1uG1uLys11eLysA1oLeuVo1G1u11eCysThrG1uMetG1uLysG1uG1yLys11eSerL
30 40 50

AGATTGGCCCCGAGAACCCCTACAACACCCCTGTGTTTGCCATCAAGAAGAAGGACTCCACCAAGTGGAGGAAGCTGGTG
ysleGlyProGluAsnProTyrAsnThrProVolPheAlolieLysLysAspSerThrLysTrpArgLysLeuVol
60 70

GACTICAGGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCCACCCCGCTGGCCTGAAGAA AspPheArgGiuLeuAsnLysArgThrGinAspPheTrpGiuVoiGinLeuGiyIieProHisProAloGiyLeuLysLy 80 90 100

GAAGAAGTCTGTGACTGTGCCGCTGTGCCCGATGCCTACTTCTCTGTGCCCCTGGATGAGGACTTCAGGAAGTACACTG slyslysSerVolThrVolLeu<u>Alo</u>VolGlyAspAloTyrPheSerVolProLeuAspG1uAspPheArgLysTyrThrA 110 120 130

CCTTCACCATCCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCAGTACAATGTGCTGCCCCAGGGCTGGAAGGGC IoPheTnrlleProSerlleAsnAsnGluThrProGlylleArgTyrGlnTyrAsnVolLeuProGlnGlyTrpLysGly 140 150

TCCCCTGCCATCTCCAGTCCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA SerProAlollePheGInSerSerMetThrLyslleLeuGiuProPheArgLysGinAsnProAsplleVoilleTyrGI 160 170 180

GTACATGGCTGCCCTGTATGTGGGCTCTGACCTGGAGATTGGGCAGCACAGGACCAAGATTGAGGAGCTGAGGCAGCACCC
nTyrMetAloAtoLeuTyrVoIGTySerAspLeuGtulleGlyGInHisArgThrLyslleGluGluLeuArgGInHisL
190 200 210

TGCTGAGGTGGGGCCTGACCACCCCTGACAAGAAGCACCAGAAGGAGCCCCCCCTTCCTGTGGATGGGCTATGAGCTGCAC euleuArgTrpGTyLeuThrThrProAsplysLysHisGInLysGTuProProPheleuTrpMetGTyTyrGTuLeuHis 220 230

CCCGACAACTGGACTGTGCACCCCATTGTGCTGCCTGAGAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG ProAspLysTrpThrVoiGinProlleVoiLeuProGluLysAspSerTrpThrVoiAsnAspIleGinLysLeuVoiGi 240 250 260

CAAGCTGAACTGGGCCTCCCAAATCTACCCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCCC yLysLeuAsnTrpAloSerGinlieTyrProGiylleLysVolArgGinLeuCysLysLeuLeuArgGiyThrLysAloL 270 280 290

#### FIGURE 17A

GGGGTGTACTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAAATCTA GiyVoiTyrTyrAspProSerLysAspLeulieAloGiulieGinLysGInGlyGInGlyGInTrpThrTyrGInlieTy 320 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGGCCCACACCAATGATGTGAAGCAGCTGA rGinGluProPheLysAsnLeuLysThrGlyLysTyrAlaArgMelArgGlyAloHisThrAsnAspVolLysGlnLeuT 350 350 370

CTCAGGCTGTGCAGAAGATCACCACTGAGTCCATTGTGATCTGGGGCAAGACCCCCAAGTTCAAGCTGCCCATCCAGAAG hrGluAloVolGinLyslleThrThrGluSerlleVollleTrpGlyLysThrProLysPheLysLeuProlleGinLys 380 390

GGTGAAGCTGTGGTACCAGCTGGAGAAGCAGCCCATTGTGGGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG uVollysleuTrpTyrGinleuGiuLysGiuProlleVolGlyAloGluThrPheTyrVolAloGlyAloAloAsnArgG 430 440 450

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCTCCCAGTATGC
LysThrAioleuGinAlolleTyrLeuAloleuGinAspSerGiyLeuGluVolAsnIieVolThrAioSerGinTyrAi
480
490
500

CCTGGGCATCATCCAGGCCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG DLeuGlyItelleGinAloGinProAspGinSerGluSerGluLeuVolAsnGinItelleGluGinLeuItelysLysG 510 520 530

AGAAGGTGTACCTGGCCTGCCCGCCCACAAGGCCATTGGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC
IULysVoITyrLeuAIoTrpVoIProAIoHisLysGIyIieGlyGIyAsnGluGInVoIAspLysLeuVoISerAIoGly
540
550

ATCAGGAACGTGCTGTTCCTGGATGGCATTGACAAGGCCCAGGATGAGCATGAGAAGTACCACTCCAACTGGAGGGCTAT

11eArgLysVolleuPheleuAspGiyIleAspLysAloGInAspGluHisGluLysTyrHisSerAsnTrpArgAloMe

560 570 580

#### FIGURE 17B

GGCCTCTGACTTCAACCTGCCCCCTGTGGTGGCTAAGGAGATTGTGCCCTCTGTGACAAGTGCCAGCTGAAGCCGGAGG tAloSerAspPheAsnLeuProProVolVolAloLysGiuileVolAloSerCysAspLysCysGinLeuLysGlyGluA 590 600 610

GCTGTGCATGTGGCCTCCGGCTACATTGAGGCTGACGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCCTGCT AlovotHisVotAloSerGlyTyrIleGluAloGluVoilleProAloGluThrGlyGlnGluThrAloTyrPheLeuLe 640 650 660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACACTGCCAATGGCTCCAACTTCACTGGGGCCACAGTGAGGGCTG uLysLeuAloGlyArgTrpProVolLysThrlleHisThrAloAsnGlySerAsnPheThrGlyAloThrVolArgAloA 680 690

CCTGCTGGTGGGCTGGCATCAAGCAGGAGTTTGGCATCCCCTACAACCCCCAGTCCCAGGGGGTGGTGGCCTCCATGAAC IoCysTrpTrpAloGlylleLysGInGluPheGlylleProTyrAsnProGInSerGinGlyVolVolAloSerMetAsn 700 710

AAGGAGCTGAAGAAGATCATTGCGCAGGTGAGGGACCAGGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTTCAT LysGluLeuLysLyslielleGlyGinVolArgAspGlnAloGluHisLeuLysThrAloVolGlnMetAloVolPhell 720 730 740

CCACAACTTCAAGAGGAAGGGGGGCATCGGGGGGCTACTCCGCTGGCGAGAGGATTGTGGACATCATTGCCACAGACATCC
eHisAsnPheLysArglysGlyGlyIleGlyGlyTyrSerAloGlyGluArglleVolAspIleIleAloThrAspIleG
750
760
770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGGTGTACTACAGGGACTCCAGGAACCCCCTGTGG
InThrLysGIuLeuGInLysGIn!!eThrLys1!eGInAsnPheArgVolTyrTyrArgAspSerArgAsnProLeuTrp
780 790

AAGGGCCCTGCCAAGCTGCTGTGGAAGGCCGAGGGGGCTGTGGTGATCCAGGACAACTCTGACATCAACGTGGTGCCCAG LysGTyProAtoLysLeuLeuTrpLysGTyGTuGTyAtoVoTVoTTeGTnAspAsnSerAspTteLysVoTVoTProAr 800 810 820

AAACCCCCCCCACATC" (SEQ ID NO: 3)
Xx Bg/ll (SEQ ID NO: 4)

FIGURE 17C

CCACCCACATCTCCCCCCATCTCCCCATTCACACTGTCCTGTCAACCTGAACCTGCCATGCC (within SEQ 10 NO: 7)
RoSerClulleSerAloProlleSerProlleCluThrValProValLysLeuLysProClyMetAspCly (within SEQ 10 NO: 8)
-1 2 70

FIGURE 18

```
- ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT
WT
                                                         -42
        - ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC GGC TGG TCC
OPT
           M G G K W S K R S V P G W S
                                                         -14
        - ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT
WT
                                                         -84
        - ACC GTG AGG GAG AGG ATG AGG AGG GCC GAG CCC GCC GCC GAC
DPT
           TVRERMRRAEPAAD
                                                         -28
        - AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA
                                                         -126
WT
        - AGG GTG AGG AGG ACC GAG CCC GCC GCC GTG GGC GTG GGC GCC
OPT
           RVRRTEPAAVGVGA
                                                         -42
        - GTA TET CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC
                                                         -168
WT
         - GTG TCC AGG GÁC CTG GÁG ÁÁG CÁC GGC GCC ÁTC ÁCC TCC TCC
OPT
          V S R D L E K H G A I T S S
                                                         -56
        - AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA
                                                         -210
WT
        - AAC ACC GCC GCC ACC AAC GCC GAC TGC GCC TGG CTG GAG GCC
OPT
           NTAATNADCAWLEA
                                                         -70
        - CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA
                                                         ·252
WT
         - CAG GAG GAC GAG GAG GTG GGC TTC CCC GTG AGG CCC CAG GTG
OPT
           O E D E E V G F P V R P Q V
                                                         -84
        - CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC
                                                         -294
WT
        - CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC
OPT
           PLRPMTYKGAVDLS
                                                         -98
        - CAC TIT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC
                                                         -336
WT
         - CAC TTC CTG AAG GAG AAG GGC GGC CTG GAG GGC CTG ATC CAC
OPT
           H F L K E K G G L E G L I H
                                                         ·112
        - TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC
                                                         -37B
WT
        - TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC S Q K R Q D I L D L W V Y H
OPT
                                                         -126
        - ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG
                                                         -420
WT
        - ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC
OPT
                                                         -140
           TOGYFPDWONYTPG
```

FIGURE 19A

WT	- CCA GGA ATC AGA TTT CCA TTG ACC TTT GGA TGG TGC TTC AAG -462	
OPT	- CCC GGC ATC AGG TTC CCC CTG ACC TTC GGC TGG TGC TTC AAG P G I R F P L T F G W C F K -154	
WT	- CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA -504	
OPT	- CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GCC AAC GAG L V P V E P E K V E E A N E ·168	
WT	- GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG -546	
OPT	- GGC GAG AAC AAC TGC CTG CTG CAC CCC ATG TCC CAG CAC GGC G E N N C L L H P M S Q H G -182	
WT	- ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC -588	
OPT	- ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC  1 E D P E K E V L E W R F D -196	)
WT .	- AGC AAG CTA GCA TTT CAT CAC GTG GCC CGA GAG CTG CAT CCG -630	١
OPT	- TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC S K L A F H H V A R E L H P -210	)
WT	- GAG TAC TAC AAG GAC TGC TGA (SEQ ID NO:30) -651	,
OPT	- GAG TAC TAC AAG GAC TGC TAA (contained within SEQ ID NO:9) E Y Y K D C (SEQ ID NO:10) -216	5

FIGURE 19B

VIJns/nef

CATGGGTCTTTTC<u>IGCAG</u>TCACCGTCCTTGAG<u>ATCI</u>GCCACC ATG GGC GGC AAG TGG TCC ANG AGG TCC GTG CCC . .

Srf1 Bg111
. . . CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGAGCAGAICIGCTGCCTTCTAGTTGCCAGC (SEQ ID NO: 38)
H P E Y Y K D C \* (contained within SEQ ID NO: 10:

V1Jns/nef(G2A.LLAA)

*Pst1*CATBAGICTTTTCIGCAGCCTCGTCGTCTTGAGATCTGCCACC ATG GCC GGC AAG TGG TCC AAG AGG TCC GTG CCC .

M A G K W S K R S V P

SrfI BallI

CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCOGGCAGAICIGCTGTGCCTTCTAATTGCCAGC (SEQ ID NO: 39)

H P E Y Y K D C \* (contained within SEQ ID NO:14)

ViJns/tpanef & ViJns/tpanef(LLAA)

Psti Catragetettticiggagteacegreettatatetaece at gat gea atg ang aga ctc tgc tgt gtg M D A M K R G L C C V

CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC GAG AIC ICC TCC AAG AGG TCC GTG CCC ...

. . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGGGGGCTGCTGCTGGCCAGC (SEQ ID NO: 40)

H P E Y Y K D C \* (contained withon seq id no: 16)

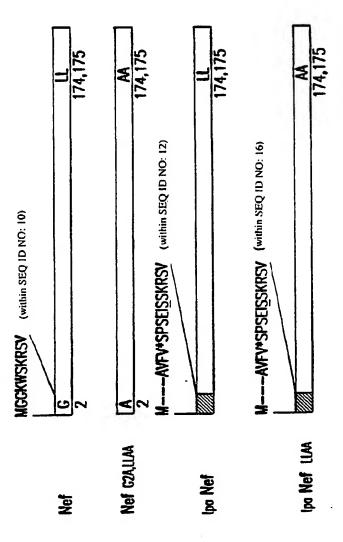


FIGURE 21

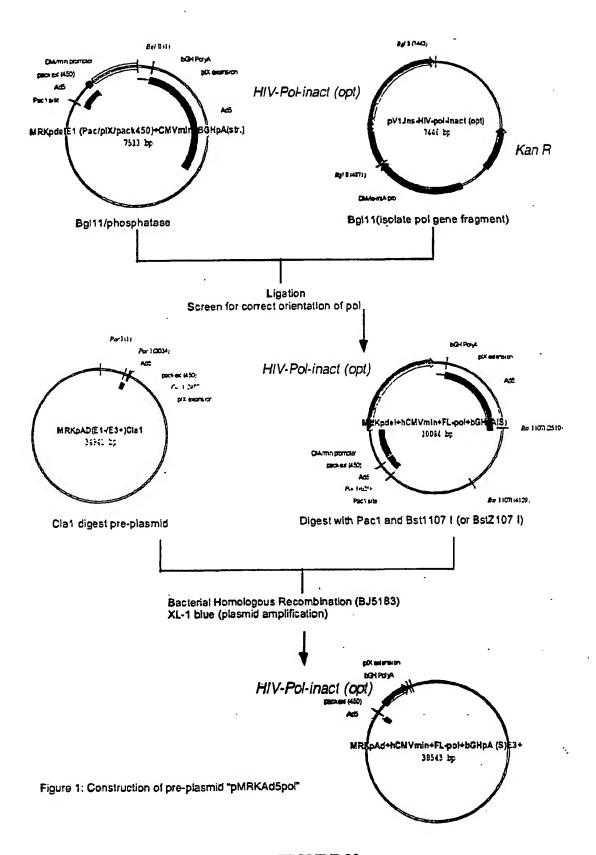


FIGURE 22

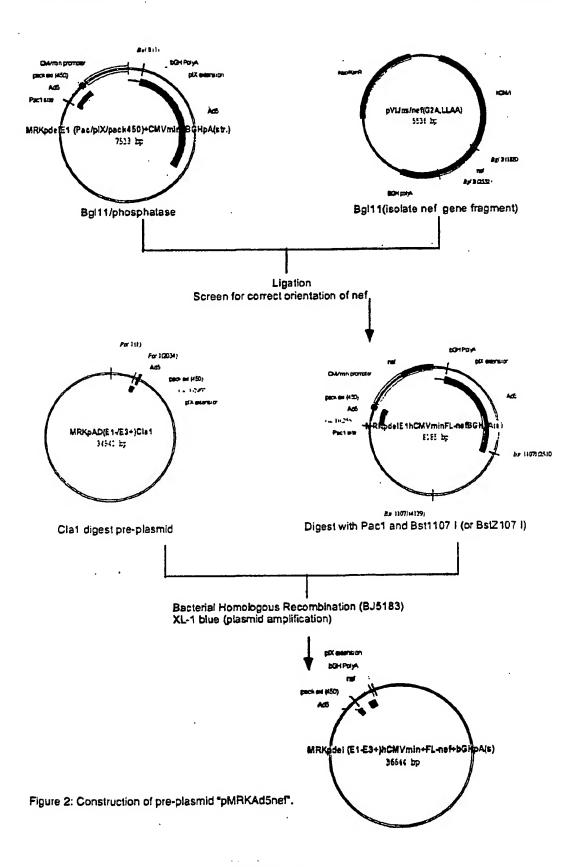


FIGURE 23

Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects

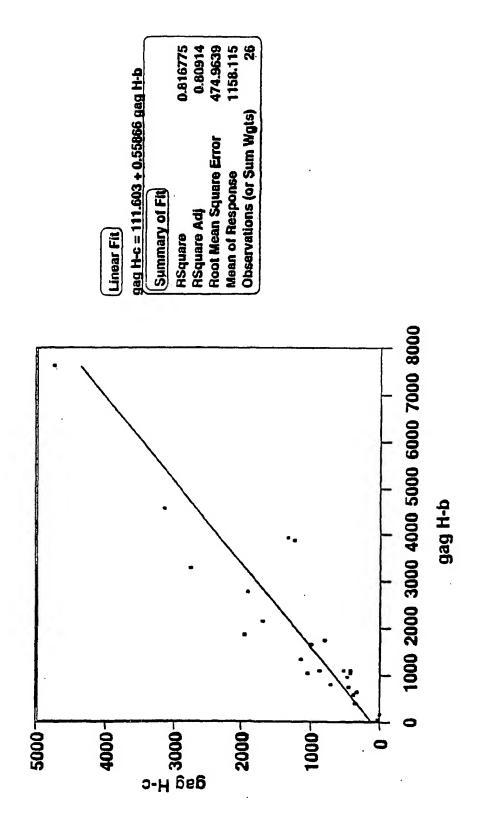
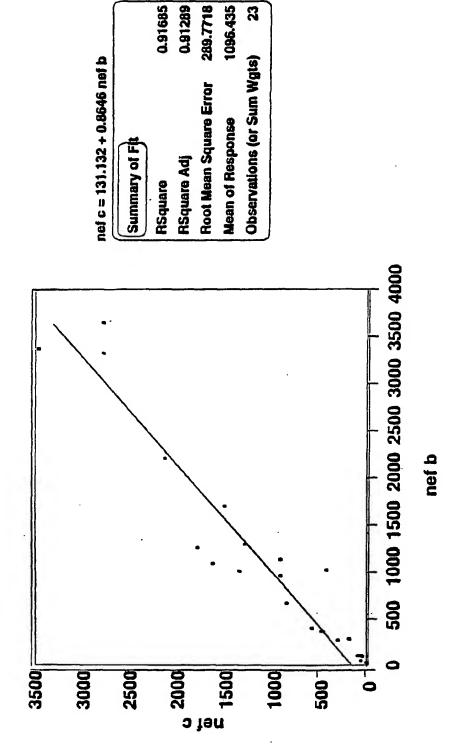


FIGURE 25

R





#### MRKAd5pol MER1062 (MRKAd5 Pre-Adenoviral Vector Containing the IA opt pol Coding Region)

1	C>0C>0C>0C>	3 3 M 3 M 3 C C M M	3 mmmmcc3 mm	CN 3 CCC 3 3 M 3	TGATAATGAG
_	•			CTTCGGTTAT	
	GIAGIAGIIA	IIAIAIGGAA	IAAAACCIAA	CIICGGIIAI	ACIAITACIC
51	GGGGTGGAGT	TTGTGACGTG	GCGCGGGGCG	TGGGAACGGG	GCGGGTGACG
	CCCCACCTCA	AACACTGCAC	CGCGCCCCGC	ACCCTTGCCC	CGCCCACTGC
101	TACTACTCTC	GCGGAAGTGT	GATGTTGCAA	GTGTGGCGGA	ACACATGTAA
				CACACCGCCT	
• • •		500011110			
151				GTGTGCGCCG	
	CGCTGCCTAC	ACCGTTTCA	CTGCAAAAAC	CACACGCGGC	CACATGTGTC
201	GAAGTGACAA	TTTTCGCGCG	GTTTTAGGCG	GATGTTGTAG	TAAATTTGGG
	CTTCACTGTT	AAAAGCGCGC	CAAAATCCGC	CTACAACATC	ATTTAAACCC
251	CGTAACCGAG	TAAGATTTGG	CCATTTTCGC	GGGAAAACTG	AATAAGAGGA
				CCCTTTTGAC	
			,		
301	AGTGAAATCT	GAATAATTTT	GTGTTACTCA	TAGCGCGTAA	TATTTGTCTA
	TCACTTTAGA	CTTATTAAAA	CACAATGAGT	ATCGCGCATT	ATAAACAGAT
351	GGGCCGCGGG	GACTTTGACC	GTTTACGTGG	AGACTCGCCC	AGGTGTTTTT
	CCCGGCGCCC	CTGAAACTGG	CAAATGCACC	TCTGAGCGGG	TCCACAAAAA
		-			
401				TTGGCGTTTT	
	GAGTCCACAA	AAGGCGCAAG	GCCCAGTTTC	AACCGCAAAA	TAATAATATC
451	GCGGCCGCGA	TCCATTGCAT	ACGTTGTATC	CATATCATAA	TATGTACATT
	CGCCGGCGCT	AGGTAACGTA	TGCAACATAG	GTATAGTATT	ATACATGTAA
501				TGTTGACATT	
	ATATAACCGA	GTACAGGTTG	TAATGGCGGT	ACAACTGTAA	CTAATAACTG
551	TAGTTATTAA	TAGTAATCAA	TTACGGGGTC	ATTAGTTCAT	AGCCCATATA
	ATCAATAATT	ATCATTAGTT	AATGCCCCAG	TAATCAAGTA	TCGGGTATAT
601	<b>ምርርኔ ር</b> ጥጥርርር	CCTTACATAA	Сттассетаа	ATGGCCCGCC	TO COMO NO CO
001				TACCGGGCGG	
		00.2.1011		2770000000	
651	CCCAACGACC	CCCGCCCATT	GACGTCAATA	ATGACGTATG	TTCCCATAGT
	GGGTTGCTGG	GGGCGGGTAA	CTGCAGTTAT	TACTGCATAC	AAGGGTATCA
701	AACGCCAATA	GGGACTTTCC	ATTGACGTCA	ATGGGTGGAG	TATTTACGGT
				TACCCACCTC	
751	AAACTGCCCA	CTTGGCAGTA	CATCAAGTGT	ATCATATGCC	AAGTACGCCC
	TTTGACGGGT	GAACCGTCAT	GTAGTTCACA	TAGTATACGG	TTCATGCGGG
801	CCTATTGACG	TCAATGACGG	TAAATGGCCC	GCCTGGCATT	ATGCCCAGTA
	GGATAACTGC				
851	CATGACCTTA				
	GTACTGGAAT	ACCCTGAAAG	GATGAACCGT	CATGTAGATG	CATAATCAGT

7 i jure 26A

901	TCGCTATTAC AGCGATAATG	GTACCACTAC	CGCTTTTGGC GCCAAAACCG	AGTACATCAA TCATGTAGTT	TGGGCG LA ACCCGCACCT
951	TAGCGGTTTG ATCGCCAAAC	ACTCACGGGG TGAGTGCCCC	ATTTCCAAGT TAAAGGTTCA	CTCCACCCCA GAGGTGGGGT	TTGACGTCAA AACTGCAGTT
1001	TGGGAGTTTG ACCCTCAAAC	TTTTGGCACC AAAACCGTGG	AAAATCAACG TTTTAGTTGC	GGACTTTCCA CCTGAAAGGT	AAATGTCGTA TTTACAGCAT
1051	TGTTGAGGCG	GGGTAACTGC	CAAATGGGCG GTTTACCCGC	CATCCGCACA	TGCCACCCTC
1101	CAGATATATT	CGTCTCGAGC	TTTAGTGAAC AAATCACTTG	GCAGTCTAGC	GGACCTCTGC
1151	GGTAGGTGCG	ACAAAACTGG	TCCATAGAAG AGGTATCTTC	TGTGGCCCTG	GCTAGGTCGG
1201	AGGCGCCGGC	CCTTGCCACG	ATTGGAACGC TAACCTTGCG	CCTAAGGGGC	ACGGTTCTCA
1251	CTCTAGATGG	TACCGGGGGT	TCTCCCCCAT AGAGGGGGTA	ACTCTGACAC	GGACACTTCG
1301	ACTTCGGACC	GTACCTACCG	CCCAAGGTGA GGGTTCCACT	TCGTCACCGG	GGACTGACTC
1351	CTCTTCTAGT	TCCGGGACCA	GGAAATCTGC CCTTTAGACG	TGACTCTACC	TCTTCCTCCC
1401	GTTTTAGAGG	TTCTAACCGG	CCGAGAACCC GGCTCTTGGG	GATGTTGTGG	GGACACAAAC
1451	GGTAGTTCTT	CTTCCTGAGG	ACCAAGTGGA TGGTTCACCT	CCTTCGACCA	CCTGAAGTCC
1501	CTCGACTTGT	TCTCCTGGGT	GGACTTCTGG CCTGAAGACC	CTCCACGTCG	ACCCGTAGGG
1551	GGTGGGGCGA	CCGGACTTCT	AGAAGAAGTC TCTTCTTCAG	ACACTGACAC	GACCGACACC
1601	CCCTACGGAT	GAAGAGACAC	CCCTGGATG GGGGACCTAC	TCCTGAAGTC	CTTCATGTGA
	CGGAAGTGGT	AGGGGAGGTA	GTTGTTACTC	TGGGGACCGT	TCAGGTACCA AGTCCATGGT
	CATGTTACAC	GACGGGGTCC	CGACCTTCCC	GAGGGGACGG	ATCTTCCAGT TAGAAGGTCA
1751	CCTCCATGAC GGAGGTACTG	CAAGATCCTG GTTCTAGGAC	GAGCCCTTCA CTCGGGAAGT	GGAAGCAGAA CCTTCGTCTT	CCCTGACATT
1801	GTGATCTACC CACTAGATGG	AGTACATGGC TCATGTACCG	TGCCCTGTAT ACGGGACATA	GTGGGCTCTG CACCCGAGAC	ACCTGGAGAT TGGACCTCTA

Figure 26B

1851	TGGGCAGCAC ACCCGTCGTG				CTGCTG T GACGACTCCA
1901		CACCCCTGAC GTGGGGACTG			
1951		ATGAGCTGCA TACTCGACGT			
2001		AAGGACTCCT TTCCTGAGGA			
2051	GCAAGCTGAA CGTTCGACTT	CTGGGCCTCC GACCCGGAGG			
2101	CTGTGCAAGC GACACGTTCG	TGCTGAGGGG ACGACTCCCC	CACCAAGGCC	CTGACTGAGG GACTGACTCC	TGATCCCCCT ACTAGGGGGA
2151	GACTGAGGAG CTGACTCCTC	GCTGAGCTGG CGACTCGACC			
2201		TGGGGTGTAC ACCCCACATG			
2251		AGGGCCAGGG TCCCGGTCCC			
2301		CTGAAGACTG GACTTCTGAC			
2351		GAAGCAGCTG CTTCGTCGAC			
2401		TCTGGGGCAA AGACCCCGTT			
2451	GGAGACCTGG CCTCTGGACC	GAGACCTGGT CTCTGGACCA	GGACTGAGTA CCTGACTCAT	CTGGCAGGCC GACCGTCCGG	ACCTGGATCC TGGACCTAGG
2501		GTTTGTGAAC CAAACACTTG			
2551	CTGGAGAAGG GACCTCTTCC	AGCCCATTGT TCGGGTAACA			
2601	TGCCAACAGG ACGGTTGTCC	GAGACCAAGC CTCTGGTTCG	TGGGCAAGGC ACCCGTTCCG	TGGCTATGTG ACCGATACAC	ACCAACAGGG TGGTTGTCCC
2651	GCAGGCAGAA CGTCCGTCTT				
2701	CTCCAGGCCA GAGGTCCGGT	TCTACCTGGC AGATGGACCG	CCTCCAGGAC GGAGGTCCTG	TCTGGCCTGG AGACCGGACC	AGGTGAACAT TCCACTTGTA
2751					CAGCCTGATC GTCGGACTAG

Figure 26 C

2801	AGTCTGAGTC TCAGACTCAG	TCTGGTG ACTCGACCAC	AACCAGATCA TTGGTCTAGT	TTGAGCAGCT AACTCGTCGA	CATCAA G CTAGTTCTTC
2851		ACCTGGCCTG TGGACCGGAC			
2901	TGAGCAGGTG ACTCGTCCAC	GACAAGCTGG CTGTTCGACC	TGTCTGCTGG ACAGACGACC	CATCAGGAAG GTAGTCCTTC	GTGCTGTTCC CACGACAAGG
2951		TGACAAGGCC ACTGTTCCGG			
3001	TGGAGGGCTA ACCTCCCGAT	TGGCCTCTGA ACCGGAGACT	CTTCAACCTG GAAGTTGGAC	CCCCTGTGG GGGGGACACC	TGGCTAAGGA ACCGATTCCT
3051	CTAACACCGG	TCCTGTGACA AGGACACTGT	TCACGGTCGA	CTTCCCCCTC	CGGTACGTAC
3101	CCGTCCACCT	CTGCTCCCCT GACGAGGGGA	CCGTAGACCG	TCGACCGGAC	GTGGGTGGAC
3151	CTCCCGTTCC	TGATCCTGGT ACTAGGACCA	CCGACACGTA	CACCGGAGGC	CGATGTAACT
3201	CCGACTCCAC	ATCCCTGCTG TAGGGACGAC	TCTGTCCGGT	CCTCTGACGG	ATGAAGGACG
3251	ACTTCGACCG	TGGCAGGTGG ACCGTCCACC	GGACACTTCT	GGTAGGTGTG	ACGGTTACCG
3301	AGGTTGAAGT	CTGGGGCCAC GACCCCGGTG	TCACTCCCGA	CGGACGACCA	CCCGACCGTA
3351	GTTCGTCCTC	TTTGGCATCC AAACCGTAGG	GGATGTTGGG	GGTCAGGGTC	CCCCACCACC
3401	GGAGGTACTT	CAAGGAGCTG GTTCCTCGAC	TTCTTCTAGT	AACCCGTCCA	CTCCCTGGTC
3451	CGACTCGTGG	TGAAGACAGC ACTTCTGTCG	ACACGTCTAC	CGACACAAGT	AGGTGTTGAA
3501	GTTCTCCTTC	CCCCCGTAGC	CCCCGATGAG	GCGACCCCTC	
	TGTAGTAACG	GTGTCTGTAG	GTCTGGTTCC	TCGAGGTCTT	GCAGATCACC CGTCTAGTGG
	TTCTAGGTCT	TGAAGTCCCA	CATGATGTCC	CTGAGGTCCT	ACCCCCTGTG TGGGGGACAC
	CTTCCCGGGA	CGGTTCGACG	ACACCTTCCC	CCTCCCCGA	GTGGTGATCC CACCACTAGG
3701	AGGACAACTC TCCTGTTGAG	TGACATCAAG ACTGTAGTTC	GTGGTGCCCA CACCACGGGT	GGAGGAAGGC CCTCCTTCCG	CAAGATCATC GTTCTAGTAG

Figure 26 D

3751	AGGGACTATG TCCCTGATAC	AGCAGAT COTTCGTCTA	GGCTGGGGAT CCGACCCCTA	GACTGTGTGGT CTGACACACC	CCTCCA CA GGAGGT GT
3801	GGATGAGGAC CCTACTCCTG	TAAAGCCCGG ATTTCGGGCC	GCAGATCTGC CGTCTAGACG	TGTGCCTTCT ACACGGAAGA	AGTTGCCAGC TCAACGGTCG
3851	CATCTGTTGT GTAGACAACA	TTGCCCCTCC AACGGGGAGG	CCCGTGCCTT GGGCACGGAA	CCTTGACCCT GGAACTGGGA	GGAAGGTGCC CCTTCCACGG
3901	ACTCCCACTG TGAGGGTGAC	TCCTTTCCTA AGGAAAGGAT	ATAAAATGAG TATTTTACTC	GAAATTGCAT CTTTAACGTA	CGCATTGTCT GCGTAACAGA
		GTAAGATAAG	ACCCCCACC	CCACCCCGTC	CTGTCGTTCC
4001	GGGAGGATTG CCCTCCTAAC	GGAAGACAAT CCTTCTGTTA	AGCAGGCATG TCGTCCGTAC	CTGGGGATGC GACCCCTACG	CCACCCGAGA
4051	ATGGCCGATC TACCGGCTAG	GGCGCGCCGT CCGCGCGGCA	ACTGAAATGT TGACTTTACA	GTGGGCGTGG CACCCGCACC	CTTAAGGGTG GAATTCCCAC
4101	CCTTTCTTAT	ATATTCCACC	CCCAGAATAC	TAGTTTTGTA ATCAAAACAT	AGACAAAACG
		CGGCGGTACT	CGTGGTTGAG	CAAACTACCT	TCGTAACACT
4201	GCTCATATTT CGAGTATAAA	GACAACGCGC CTGTTGCGCG	ATGCCCCCAT TACGGGGGTA	GGGCCGGGGT CCCGGCCCCA	GCGTCAGAAT CGCAGTCTTA
4251		GGTCGTAACT	ACCAGCGGGG	CAGGACGGGC	GTTTGAGATG
4301	ATGGAACTGG	ATGCTCTGGC	ACAGACCTTG	GCCGTTGGAG CGGCAACCTC	TGACGTCGGA
4351	GCCGCCGCCG	AAGTCGGCGA	CGTCGGTGGC	CCCGCGGGAT GGGCGCCCTA	ACACTGACTG
4401	AAACGAAAGG	ACTCGGGCGA	ACGTTTGTCA	GCAGCTTCCC CGTCGAAGGG	CAAGTAGGCG
4451	GGCGCTACTG	TTCAACTGCC	GAGAAAACCG	ACAATTGGAT TGTTAACCTA	AGAAACTGGG
	_	ACAGCAAAGA	GTCGTCGACA	ACCTAGACGC	GGTCGTCCAA
		TCCGAAGGAG	GGGAGGGTTA	CGCCAAATTT	TGTATTTATT
		AGACAAACCT	AAACCTAGTT	CGTTCACAGA	ACGACAGAAA
4651	ATTTAGGGGT TAAATCCCCA	TTTGCGCGCG AAACGCGCGC	CGGTAGGCCC GCCATCCGGG	GGGACCAGCG CCCTGGTCGC	GTCTCGGTCG CAGAGCCAGC

Figure 26E

4701	TTGAGGGTCC AACTCCCAGG	TCTGTATTTT ATAAAA	TTCCAGGACG AAGGTCCTGC	TGGTAAAGGT <sup>L</sup> ACCATTTCCA	-GACTCTCEAT CTGAGA A
4751				GGGGTGGAGG CCCCACCTCC	
4801				AGATGATCCA TCTACTAGGT	
4851				TTCAGTAGCA AAGTCATCGT	
4901				AAAGCGGTTA TTTCGCCAAT	
4951				TGGACTGTAT ACCTGACATA	
5001				TTCATGTTGT AAGTACAACA	
5051	CAGCACAGTG GTCGTGTCAC	TATCCGGTGC ATAGGCCACG	ACTTGGGAAA TGAACCCTTT	TTTGTCATGT AAACAGTACA	AGCTTAGAAG TCGAATCTTC
5101				TGTGACCTCC ACACTGGAGG	
5151				CCACGGGCGG GGTGCCCGCC	
5201	GAAGATATTT C'ITCTATAAA	CTGGGATCAC GACCCTAGTG	TAACGTCATA ATTGCAGTAT	GTTGTGTTCC CAACACAAGG	AGGATGAGAT TCCTACTCTA
5251				GGAGGGTGCC CCTCCCACGG	
5301				TTACCCTCAC AATGGGAGTG	
5351				CATGTCTACC GTACAGATGG	
5401	TGAAGAAAAC ACTTCTTTTG	GGTTTCCGGG CCAAAGGCCC	GTAGGGGAGA CATCCCCTCT	TCAGCTGGGA AGTCGACCCT	AGAAAGCAGG TCTTTCGTCC
5451	TTCCTGAGCA AAGGACTCGT	GCTGCGACTT CGACGCTGAA	ACCGCAGCCG TGGCGTCGGC	GTGGGCCCGT CACCCGGGCA	AAATCACACC TTTAGTGTGG
5501	TATTACCGGC ATAATGGCCG			GCTGCAGCTG CGACGTCGAC	
5551	TGAGCAGGGG ACTCGTCCCC	GGCCACTTCG CCGGTGAAGC	TTAAGCATGT AATTCGTACA	CCCTGACTCG GGGACTGAGC	CATGTTTTCC GTACAAAAGG
5601	CTGACCAAAT GACTGGTTTA	CCGCCAGAAG GGCGGTCTTC	GCGCTCGCCG CGCGAGCGGC	CCCAGCGATA GGGTCGCTAT	GCAGTTCTTG CGTCAAGAAC

Figure 26 F

5651	CAAGGAAGCA GTTCCTTCGT			ACCGTCCGCC TGGCAGGCGG	
5701	TTTTGAGCGT	TTGACCAAGC	AGTTCCAGGC	GGTCCCACAG	CTCGGTCACC
	AAAACTCGCA	AACTGGTTCG	TCAAGGTCCG	CCAGGGTGTC	GAGCCAGTGG
5751				CCTCGTTTCG GGAGCAAAGC	
5801	CGGCTTTCGC	TGTACGGCAG	TAGTCGGTGC	TCGTCCAGAC	GGGCCAGGGT
	GCCGAAAGCG	ACATGCCGTC	ATCAGCCACG	AGCAGGTCTG	CCCGGTCCCA
5851	CATGTCTTTC GTACAGAAAG			CAGCGTAGTC GTCGCATCAG	
5901				CCAGGGTGCG GGTCCCACGC	
5951				TCGCCCTGCG AGCGGGACGC	
6001	GTAGCATTTG	ACCATGGTGT	CATAGTCCAG	CCCCTCCGCG	GCGTGGCCCT
	CATCGTAAAC	TGGTACCACA	GTATCAGGTC	GGGGAGGCGC	CGCACCGGGA
6051				CGCACGAGGG GCGTGCTCCC	
6101	CTTTTGAGGG	CGTAGAGCTT	GGGCGCGAGA	AATACCGATT	CCGGGGAGTA
	GAAAACTCCC	GCATCTCGAA	CCCGCGCTCT	TTATGGCTAA	GGCCCCTCAT
6151				CTCGCATTCC GAGCGTAAGG	
6201				GGTTTCCCCC CCAAAGGGGG	
6251	ATGCGTTTCT	TACCTCTGGT	TTCCATGAGC	CGGTGTCCAC	GCTCGGTGAC
	TACGCAAAGA	ATGGAGACCA	AAGGTACTCG	GCCACAGGTG	CGAGCCACTG
6301				CTTGAGAGGC GAACTCTCCG	
6351	GCGGTGTTCC	GCGGTCCTCC	TCGTATAGAA	ACTCGGACCA	CTCTGAGACA
	CGCCACAAGG	CGCCAGGAGG	AGCATATCTT	TGAGCCTGGT	GAGACTCTGT
6401	AAGGCTCGCG	TCCAGGCCAG	CACGAAGGAG	GCTAAGTGGG	AGGGGTAGCG
	TTCCGAGCGC	AGGTCCGGTC	GTGCTTCCTC	CGATTCACCC	TCCCCATCGC
6451	GTCGTTGTCC	ACTAGGGGGT	CCACTCGCTC	CAGGGTGTGA	AGACACATGT
	CAGCAACAGG	TGATCCCCCA	GGTGAGCGAG	GTCCCACACT	TCTGTGTACA
6501	CGCCCTCTTC	GGCATCAAGG	AAGGTGATTG	GTTTGTAGGT	GTAGGCCACG
	GCGGGAGAAG	CCGTAGTTCC	TTCCACTAAC	CAAACATCCA	CATCCGGTGC
6551	TGACCGGGTG	TTCCTGAAGG	GGGGCTATAA	AAGGGGGTGG	GGGCGCGTTC
	ACTGGCCCAC	AAGGACTTCC	CCCCGATATT	TTCCCCCACC	CCCGCGCAAG

Figure 266

6601	GTCCTCACTC CAGGAGTGAG	TCTTCCGCAT AGGCGTA	CGCTGTCTGC GCGACAGACG	CAGGGCCAGE CTCCCGGTCG	ACAACO .C
6651	AGTACTCCCT	CTGAAAAGCG	GGCATGACTT	CTGCGCTAAG	ATTGTCAGTT
	TCATGAGGGA	GACTTTTCGC	CCGTACTGAA	GACGCGATTC	TAACAGTCAA
6701	TCCAAAAACG	AGGAGGATTT	GATATTCACC	TGGCCCGCGG	TGATGCCTTT
	AGGTTTTTGC	TCCTCCTAAA	CTATAAGTGG	ACCGGGCGCC	ACTACGGAAA
6751	GAGGGTGGCC	GCATCCATCT	GGTCAGAAAA	GACAATCTTT	TTGTTGTCAA
	CTCCCACCGG	CGTAGGTAGA	CCAGTCTTTT	CTGTTAGAAA	AACAACAGTT
6801	GCTTGGTGGC	AAACGACCCG	TAGAGGGCGT	TGGACAGCAA	CTTGGCGATG
	CGAACCACCG	TTTGCTGGGC	ATCTCCCGCA	ACCTGTCGTT	GAACCGCTAC
6851	GAGCGCAGGG	TTTGGTTTTT	GTCGCGATCG	GCGCGCTCCT	TGGCCGCGAT
	CTCGCGTCCC	AAACCAAAAA	CAGCGCTAGC	CGCGCGAGGA	ACCGGCGCTA
6901	GTTTAGCTGC	ACGTATTCGC	GCGCAACGCA	CCGCCATTCG	GGAAAGACGG
	CAAATCGACG	TGCATAAGCG	CGCGTTGCGT	GGCGGTAAGC	CCTTTCTGCC
6951	TGGTGCGCTC	GTCGGGCACC	AGGTGCACGC	GCCAACCGCG	GTTGTGCAGG
	ACCACGCGAG	CAGCCCGTGG	TCCACGTGCG	CGGTTGGCGC	CAACACGTCC
7001	GTGACAAGGT	CAACGCTGGT	GGCTACCTCT	CCGCGTAGGC	GCTCGTTGGT
	CACTGTTCCA	GTTGCGACCA	CCGATGGAGA	GGCGCATCCG	CGAGCAACCA
7051	CCAGCAGAGG	CGGCCGCCCT	TGCGCGAGCA	GAATGGCGGT	AGGGGGTCTA
	GGTĆGTCTCC	GCCGGCGGGA	ACGCGCTCGT	CTTACCGCCA	TCCCCCAGAT
7101	GCTGCGTCTC	GTCCGGGGGG	TCTGCGTCCA	CGGTAAAGAC	CCCGGGCAGC
	CGACGCAGAG	CAGGCCCCCC	AGACGCAGGT	GCCATTTCTG	GGGCCCGTCG
7151	TCCGCGCGCA	GCTTCATCAG	ATAGAACGTA	CCTTGCAAGT GGAACGTTCA	GATCGCGGAC
7201	GACGGTACGC	GCCCGCCGTT	CGCGCGCGAG	GTATGGGTTG CATACCCAAC	TCACCCCCTG
7251	GGGTACCGTA	CCCCACCCAC	TCGCGCCTCC	CGTACATGCC GCATGTACGG	CGTTTACAGC
7301	ATTTGCATCT	CCCCGAGAGA	CTCATAAGGT	AGATATGTAG TCTATACATC	CCATCGTAGA
		TACGACCGCG	CGTGCATTAG	CATATCAAGC	ACGCTCCCTC
		CCCTGGCTCC	AACGATGCCC	GCCCGACGAG	ACGAGCCTTC
7451	ACTATCTGCC	TGAAGATGGC	ATGTGAGTTG	GATGATATGG	TTGGACGCTG
	TGATAGACGG	ACTTCTACCG	TACACTCAAC	CTACTATACC	AACCTGCGAC
7501	GAAGACGTTG	AAGCTGGCGT	CTGTGAGACC	TACCGCGTCA	CGCACGAAGG
	CTTCTGCAAC	TTCGACCGCA	GACACTCTGG	ATGGCGCAGT	GCGTGCTTCC

Figure 26 H

7551		GCGCGCGTCG			
7601		AGTAGTCCAG TCATCAGGTC			
7651		TTCCACAGCT AAGGTGTCGA			_
7701		TTGGATCGGA AACCTAGCCT			
7751		ACTGGTTGAC TGACCAACTG			
7801		TATGCCTGCG ATACGGACGC			
7851		CCTGACCATG GGACTGGTAC			
7901		CGCCCTGCTC GCGGGACGAG			
7951		GGCAGGGCGA CCGTCCCGCT			
8001		AAAGTTGCGT TTTCAACGCA			
8051		TTACCTGGGC AATGGACCCG			
8101		ACAATGTAAA TGTTACATTT			
8151		TTTAAGTTCC AAATTCAAGG			
8201		AAAGGGCCCA TTTCCCGGGT			
8251		AGGTCACGGG TCCAGTGCCC			
8301	TCCTAAACTG AGGATTTGAC	GCGACCTATG CGCTGGATAC	GCCATTTTTT CGGTAAAAA	CTGGGGTGAT GACCCCACTA	GCAGTAGAAG CGTCATCTTC
8351	GTAAGCGGGT CATTCGCCCA				CGGCTAGGTC GCCGATCCAG
8401	TCGCGCGGCA AGCGCGCGT	GTCACTAGAG CAGTGATCTC	GCTCATCTCC CGAGTAGAGG	GCCGAACTTC CGGCTTGAAG	ATGACCAGCA TACTGGTCGT
8451	TGAAGGGCAC ACTTCCCGTG				ATAGGTCTCT TATCCAGAGA

Figure 26I

8501	ACATCGTAGG TGTAGCATCC	TAAAGAG ACTGTTTCTC	ACGCTCGGTG TGCGAGCCAC	CGAGGATGCG GCTCCTACGC	AGCCGA G TCGGCTAGCC
8551	GAAGAACTGG CTTCTTGACC	ATCTCCCGCC TAGAGGGCGG	ACCAATTGGA TGGTTAACCT	GGAGTGGCTA CCTCACCGAT	TTGATGTGGT AACTACACCA
8601	CTTTCATCTT	GTCCCTGCGA CAGGGACGCT	GCCCGCCTTG	TGAGCACGAC	CGAAAACATT
8651	TTTGCACGCG	AGTACTGGCA TCATGACCGT	CGCCACGTGC	CCGACATGTA	GGACGTGCTC
8701	CAACTGGACT	CGACCGCGCA GCTGGCGCGT	GTTCCTTCGT	CTCACCCTTA	AACTCGGGGA
8751	GCGGACCGCC	CTTTGGCTGG CAAACCGACC	ACCAGAAGAT	GAAGCCGACG	AACAGGAACT
8801	GGCAGACCGA	GCTCGAGGGG CGAGCTCCCC	TCAATGCCAC	CTAGCCTGGT	GGTGCGGCGC
8851	GCTCGGGTTT	GTCCAGATGT CAGGTCTACA	GGCGCGCCC	GCCAGCCTCG	AACTACTGTT
8901	GTAGCGCGTC	ATGGGAGCTG TACCCTCGAC	AGGTACCAGA	CCTCGAGGGC	GCCGCAGTCC
8951	AGTCCGCCCT	GCTCCTGCAG CGAGGACGTC	CAAATGGAGC	GTATCTGCCC	AGTCCCGCGC
9001	CCGATCTAGG	AGGTGATACC TCCACTATGG	ATTAAAGGTC	CCCGACCAAC	CACCGCCGCA
9051	GCTACCGAAC	CAAGAGGCCG GTTCTCCGGC	GTAGGGGCGC	CGCGCTGATG	CCATGGCGCG
9101	CCGCCCGCCA	CCCGCCGCCCC	CCACAGGAAC	CTACTACGTA	GATTTTCGCC
9151	ACTGCGCCCG	GAGCCCCCGG CTCGGGGGCC	TCCATCCCC	CCGAGGCCTG	GGCGGCCCTC
9201	TCCCCCGTCC	CCGTGCAGCC	GCGGCGCGCG	CCCGTCCTCG	
	GCGCATCCAA	CGACCGCTTG	CGCTGCTGCG	CCGCCAACTA	CTCCTGAATC GAGGACTTAG
	ACCGCGGAGA	CGCACTTCTG	CTGCCCGGGC	CACTCGAACT	ACCTGAAAGA TGGACTTTCT
	CTCAAGCTGT	CTTAGTTAAA	. GCCACAGCAA	CTGCCGCCGG	TGGCGCAAAA ACCGCGTTTT
9401	TCTCCTGCAC AGAGGACGTG	GTCTCCTGAG CAGAGGACTC	TTGTCTTGAT	AGGCGATCTC TCCGCTAGAG	GGCCATGAAC

Figure 26 J

9451		CTCCTG GAAGGAGGAC			
9501	GGCGGCGAGG CCGCCGCTCC	TCGTTGGAAA AGCAACCTTT			
9551		GTTCCAGACG CAAGGTCTGC			
9601		TGACCACCTG ACTGGTGGAC			
9651	GACGGCGTAG CTGCCGCATC	TTTCGCAGGC AAAGCGTCCG			
9701	ACACAAGACG	CACGAAGAAG GIGCTTCTTC	ATGTATTGGG	TCGCAGCGTT	GCACCTAAGC
9751	AACTATAGGG	CCAAGGCCTC GGTTCCGGAG	TTCCGCGAGG	TACCGGAGCA	TCTTCAGGTG
9801	CCGCTTCAAC	AAAAACTGGG TTTTTGACCC	TCAACGCGCG	GCTGTGCCAA	TTGAGGAGGA
9851	GGTCTTCTGC	GATGAGCTCG CTACTCGAGC	CGCTGTCACA	GCGCGTGGAG	CGCGAGTTTC
9901	CGATGTCCCC	CCTCTTCTTC GGAGAAGAAG	AAGAAGTTAG	AGGAGAAGGT	ATTCCCGGAG
9951	GGGAAGAAGA	TCTTCTGGCG AGAAGACCGC	CGCCACCCCC	TCCCCCTGT	GCCGCCGCTG
10001	CTGCCGCGTG	CGGGAGGCGG GCCCTCCGCC	AGCTGTTTCG	CGAGCTAGTA	GAGGGGCGCC
10051	GCTGCCGCGT	TGGTCTCGGT ACCAGAGCCA	CTGCCGCGCC	GGCAAGAGCG	CCCCGCGTC
10101	AACCTTCTGC	CCGCCCGTCA GGCGGGCAGT	ACAGGGCCAA	TACCCAACCG	CCCCCGACG
		CCTATGCCGC	GATTGCTACG	TAGAGTTGTT	AACAACACAT
		GCGGCTCCCT	GGACTCGCTC	AGGCGTAGCT	GGCCTAGCCT
		TCTTTCCGCA	GATTGGTCAG	TGTCAGCGTT	CCATCCGACT
		CCCGCCGTCG	CCCGCCGCCA	GCCCCAACAA	AGACCGCCTC
10351	GTGCTGCTGA CACGACGACT	TGATGTAATT ACTACATTAA	AAAGTAGGCG TTTCATCCGC	GTCTTGAGAC CAGAACTCTG	GGCGGATGGT

Figure 26 K

10401	CGACAGAAGC GCTGTCTTCG	A TGTCCT TACAGGA	TGGGTCCGGC ACCCAGGCCG	CTGCTGAATG GACGACTTAC	CGCAGG T GCGTCCCA
10451	CGGCCATGCC GCCGGTACGG	CCAGGCTTCG GGTCCGAAGC	TTTTGACATC AAAACTGTAG	GGCGCAGGTC CCGCGTCCAG	TTTGTAGTAG AAACATCATC
10501	TCTTGCATGA AGAACGTACT	GCCTTTCTAC CGGAAAGATG	CGGCACTTCT GCCGTGAAGA	TCTTCTCCTT AGAAGAGGAA	CCTCTTGTCC GGAGAACAGG
10551		CGTAGATAGC	GACGCCGCCG	CCGCCTCAAA	CCGGCATCCA
10601	CCGCGGGAGA	AGGAGGGTAC	GCACACTGGG		GTAGCCGACT
10651	TCGTCCCGAT	CCAGCCGCTG	TTGCGCGAGC	GCTAATATGG CGATTATACC	GGACGACGTG
10701	GACGCACTCC	CATCTGACCT	TCAGTAGGTA	GTCCACAAAG CAGGTGTTTC	GCCACCATAC
10751	GCGGGCACAA	CTACCACATT	CACGTCAACC	CCATAACGGA GGTATTGCCT	GGTCAATTGC
10801	CAGACCACTG	GGCCGACGCT	CTCGAGCCAC	TACCTGAGAC ATGGACTCTG	CGCTCATTCG
10851	CCTCGAGTCA GGAGCTCAGT	AATACGTAGT TTATGCATCA	CGTTGCAAGT GCAACGTTCA	CCGCACCAGG GGCGTGGTCC	TACTGGTATC ATGACCATAG
10901	GGTGGTTTTT	CACGCCGCCG	CCGACCGCCA	AGAGGGGCCA TCTCCCCGGT	CGEATCCCAC
10951	CGGCCCCGAG	GCCCCGCTC	TAGAAGGTTG	TATTCCGCTA	
11001	CTACATGGAC	CTGTAGGTCC	ACTACGGCCG	GGCGGTGGTG	CTCCGCGCGC
11051	CTTTCAGCGC	CTGCGCCAAG	GTCTACAACG	GCAGCGGCAA CGTCGCCGTT	TTTCACGAGG
11101	TACCAGCCCT	GCGAGACCGG	CCAGTCCGCG	. CGCGTTAGCA	TGACGCTCTA ACTGCGAGAT
11151	GACCGTGCAA CTGGCACGTT	AAGGAGAGCC TTCCTCTCGG	TGTAAGCGGG ACATTCGCCC	CACTCTTCCG GTGAGAAGGC	TGGTCTGGTG ACCAGACCAC
	CTATTTAAGC	GTTCCCATAG	TACCGCCTGC	TGGCCCCAAG	GAGCCCCGTA CTCGGGGCAT
11251	TCCGGCCGTC AGGCCGGCAG	CGCCGTGATC GCGGCACTAG	CATGCGGTTA GTACGCCAAT	CCGCCGCGT	GTCGAACCCA CAGCTTGGGT
11301	GCTCTGCGAC CCACACGCTG	GTCAGACAAC CAGTCTGTTG	GGGGGAGTGC CCCCTCACG	TCCTTTTGGC AGGAAAACCG	TTCCTTCCAG AAGGAAGGTC

Figure 26L

11351	CGCGCCGCCGC	T TGCGCTA ACGACGCGAT	GCTTTTTTGG CGAAAAAACC	CCACTGGCCG GGTGACCGGC	CGCGCA ST GCGCGTCCA
11401	AAGCGGTTAG TTCGCCAATC			AGTGGCTCGC TCACCGAGCG	
11451	GCCTCCCAAT	AAAAGGTTCC	CAACTCAGCG	GGGACCCCCG CCCTGGGGGC	CAAGCTCAGA
11501				TTGCCTCCCC AACGGAGGGG	
11551	TGGGGCGAAC	GTTTAAGGAG	GCCTTTGTCC	GACGAGCCCC CTGCTCGGGG	AAAAAACGAA
11601				GCGCCCCCT	
11651				GGGCACCCTC CCCGTGGGAG	
11701				GACGCGGCAG CTGCGCCGTC	
11751	AATGCTTGGG	GCCCCCCCG	CCCGGGCCGT	CTACCTGGAC GATGGACCTG	AACCTCCTCC
11801	CGCTCCCGGA	CCGCGCCGAT	CCTCGCGGGA	CTCCTGAGCG GAGGACTCGC	CGTGGGTTCC
11851				TACGTGCCGC ATGCACGGCG	
11901				GGAGATGCGG CCTCTACGCC	
11951				TGAATCGCGA ACTTAGCGCT	
12001				ACCGGGATTA TGGCCCTAAT	
12051	GCGTGTGCAC	Cecceccec	TGGACCATTG	CGCATACGAG GCGTATGCTC	GTCTGCCACT
12101	ACCAGGAGAT TGGTCCTCTA	TAACTTTCAA ATTGAAAGTT	AAAAGCTTTA TTTTCGAAAT	ACAACCACGT TGTTGGTGCA	GCGTACGCTT CGCATGCGAA
12151	GTGGCGCGCG CACCGCGCGC	AGGAGGTGGC TCCTCCACCG	TATAGGACTG ATATCCTGAC	ATGCATCTGT TACGTAGACA	GGGACTTTGT CCCTGAAACA
12201	AAGCGCGCTG TTCGCGCGAC				GCGCAGCTGT CGCGTCGACA
12251	TCCTTATAGT AGGAATATCA			AGGCATTCAG TCCGTAAGTC	

7 igure 26 M

12301	CTAAACATAG	T GCCCGA	GGGCCGCTGG	CTGCTCGATT	TGATAA TT
	GATTTGTATC	ATCTCGGGCT	CCCGGCGACC	GACGAGCTAA	ACTATTTGTA
12351	CCTGCAGAGC	ATAGTGGTGC	AGGAGCGCAG	CTTGAGCCTG	GCTGACAAGG
	GGACGTCTCG	TATCACCACG	TCCTCGCGTC	GAACTCGGAC	CGACTGTTCC
12401	TGGCCGCCAT	CAACTATTCC	ATGCTTAGCC	TGGGCAAGTT	TTACGCCCGC
	ACCGGCGGTA	GTTGATAAGG	TACGAATCGG	ACCCGTTCAA	AATGCGGGCG
12451	AAGATATACC TTCTATATGG	ATACCCCTTA TATGGGGAAT	CGTTCCCATA GCAAGGGTAT	GACAAGGAGG CTGTTCCTCC	TAAAGATCGA ATTTCTAGCT
12501	GGGGTTCTAC	ATGCGCATGG	CGCTGAAGGT	GCTTACCTTG	AGCGACGACC
	CCCCAAGATG	TACGCGTACC	GCGACTTCCA	CGAATGGAAC	TCGCTGCTGG
12551	ACCCGCAAAT	AGCGTTGCTC	CGCATCCACA GCGTAGGTGT	TCCGGCACTC	GCACTCGGCC
12601	CGGCGCGAGC	TCAGCGACCG	CGAGCTGATG	CACAGCCTGC	AAAGGGCCCT
	GCCGCGCTCG	AGTCGCTGGC	GCTCGACTAC	GTGTCGGACG	TTTCCCGGGA
12651	GGCTGGCACG	GGCAGCGGCG	ATAGAGAGGC	CGAGTCCTAC	TTTGACGCGG
	CCGACCGTGC	CCGTCGCCGC	TATCTCTCCG	GCTCAGGATG	AAACTGCGCC
12701	GCGCTGACCT	GCGCTGGGCC	CCAAGCCGAC	GCGCCCTGGA	GGCAGCTGGG
	CGCGACTGGA	CGCGACCCGG	GGTTCGGCTG	CGCGGGACCT	CCGTCGACCC
12751	GCCGGACCTG	GGCTGGCGGT	GGCACCCGCG	CGCGCTGGCA	ACGTCGGCGG
	CGGCCTGGAC	CCGACCGCCA	CCGTGGGCGC	GCGCGACCGT	TGCAGCCGCC
12801	CGTGGAGGAA	TATGACGAGG	ACGATGAGTA	CGAGCCAGAG	GACGGCGAGT
	GCACCTCCTT	ATACTGCTCC	TGCTACTCAT	GCTCGGTCTC	CTGCCGCTCA
12851	ACTAAGCGGT	GATGTTTCTG	ATCAGATGAT	GCAAGACGCA	ACGGACCCGG
	TGATTCGCCA	CTACAAAGAC	TAGTCTACTA	CGTTCTGCGT	TGCCTGGGCC
12901	CGGTGCGGGC	GGCGCTGCAG	AGCCAGCCGT	CCGGCCTTAA	CTCCACGGAC
	GCCACGCCCG	CCGCGACGTC	TCGGTCGGCA	GGCCGGAATT	GAGGTGCCTG
12951	GACTGGCGCC	AGGTCATGGA	CCGCATCATG	TCGCTGACTG	CGCGCAATCC
	CTGACCGCGG	TCCAGTACCT	GGCGTAGTAC	AGCGACTGAC	GCGCGTTAGG
13001	TGACGCGTTC	CGGCAGCAGC	CGCAGGCCAA	CCGGCTCTCC	GCAATTCTGG
	ACTGCGCAAG	GCCGTCGTCG	GCGTCCGGTT	GGCCGAGAGG	CGTTAAGACC
13051	AAGCGGTGGT TTCGCCACCA	CCCGGCGCGC	GCAAACCCCA CGTTTGGGGT	CGCACGAGAA GCGTGCTCTT	GGTGCTGGCG CCACGACCGC
13101	ATCGTAAACG	CGCTGGCCGA	AAACAGGGCC	ATCCGGCCCG	ACGAGGCCGG
	TAGCATTTGC	GCGACCGGCT	TTTGTCCCGG	TAGGCCGGGC	TGCTCCGGCC
13151	CCTGGTCTAC	GACGCGCTGC	TTCAGCGCGT	GGCTCGTTAC	AACAGCGGCA
	GGACCAGATG	CTGCGCGACG	AAGTCGCGCA	CCGAGCAATG	TTGTCGCCGT
13201	ACGTGCAGAC	CAACCTGGAC	CGGCTGGTGG	GGGATGTGCG	CGAGGCCGTG
	TGCACGTCTG	GTTGGACCTG	GCCGACCACC	CCCTACACGC	GCTCCGGCAC

Figure 26 N.

13251			GCAGCAGGGC CGTCGTCCCG		
13301			CACAGCCCGC GTGTCGGGCG		
13351			AGCGCACTGC TCGCGTGACG		
13401			GTCTGGGCCA CAGACCCGGT		
13451			TAAACCTGAG ATTTGGACTC		
13501	AGGGGCTGTG TCCCCGACAC	GGGGGTGCGG CCCCCACGCC	GCTCCCACAG CGAGGGTGTC	GCGACCGCGC CCCTGCCGCG	GACCGTGTCT CTGGCACAGA
13551			GCGCCTGTTG CGCGGACAAC		
13601			CCCGGGACAC GGGCCCTGTG		
13651			GGTCAGGCGC CCAGTCCGCG		
13701			CCGCGCGCTG GGCGCGCGAC		
13751			ACCTGCTGAC TGGACGACTG		
13801			AGCGAGGAGG TCGCTCCTCC		
13851			CCTGATGCGC GGACTACGCG		
13901	GGCGCTGGAC CCGCGACCTG	ATGACCGCGC TACTGGCGCG	GCAACATGGA CGTTGTACCT	ACCGGGCATG TGGCCCGTAC	TATGCCTCAA ATACGGAGTT
13951			CTAATGGACT GATTACCTGA		
14001	GTGAACCCCG CACTTGGGGC	AGTATTTCAC TCATAAAGTG	CAATGCCATC GTTACGGTAG	TTGAACCCGC AACTTGGGCG	ACTGGCTACC TGACCGATGG
14051	GCCCCTGGT CGGGGGACCA	TTCTACACCG AAGATGTGGC	GGGGATTCGA CCCCTAAGCT	GGTGCCCGAG CCACGGGCTC	GCTAACGATG CCATTGCTAC
14101	GATTCCTCTG CTAAGGAGAC	GGACGACATA CCTGCTGTAT	GACGACAGCG CTGCTGTCGC	TGTTTTCCCC ACAAAAGGGG	GCAACCGCAG CGTTGGCGTC
14151	ACCCTGCTAG TGGGACGATC	AGTTGCAACA TCAACGTTGT	GCGCGAGCAG CGCGCTCGTC	GCAGAGGCGG CGTCTCCGCC	CGCTGCGAAA GCGACGCTTT

7, gure 260

14201	GGAAAGCTTC CCTTTCGAAG	CORRECCAA	GCAGCTTGTC CGTCGAACAG	CGATCTAGGC <sup>*</sup> GCTAGATCCG	CGACGC G
14251	CGCGGTCAGA	TGCTAGTAGC	CCATTTCCAA	GCTTGATAGG	GTCTCTTACC
	GCGCCAGTCT	ACGATCATCG	GGTAAAGGTT	CGAACTATCC	CAGAGAATGG
14301	AGCACTCGCA	CCACCCGCCC	GCGCCTGCTG	GGCGAGGAGG	AGTACCTAAA
	TCGTGAGCGT	GGTGGGCGGG	CGCGGACGAC	CCGCTCCTCC	TCATGGATTT
14351	CAACTCGCTG	CTGCAGCCGC	AGCGCGAAAA	AAACCTGCCT	CCGGCATTTC
	GTTGAGCGAC	GACGTCGGCG	TCGCGCTTTT	TTTGGACGGA	GGCCGTAAAG
14401	CCAACAACGG	GATAGAGAGC	CTAGTGGACA	AGATGAGTAG	ATGGAAGACG
	GGTTGTTGCC	CTATCTCTCG	GATCACCTGT	TCTACTCATC	TACCTTCTGC
14451	TACGCGCAGG ATGCGCGTCC	AGCACAGGGA TCGTGTCCCT	CGTGCCAGGC GCACGGTCCG	GCGCGCGCGC	CCACCCGTCG GGTGGGCAGC
14501	TCAAAGGCAC	GACCGTCAGC	GGGGTCTGGT	GTGGGAGGAC	GATGACTCGG
	AGTTTCCGTG	CTGGCAGTCG	CCCCAGACCA	CACCCTCCTG	CTACTGAGCC
14551	CAGACGACAG	CAGCGTCCTG	GATTTGGGAG	GGAGTGGCAA	CCCGTTTGCG
	GTCTGCTGTC	GTCGCAGGAC	CTAAACCCTC	CCTCACCGTT	GGGCAAACGC
14601	CACCTTCGCC	CCAGGCTGGG	GAGAATGTTT	TAAAAAAAAA	AAAAGCATGA
	GTGGAAGCGG	GGTCCGACCC	CTCTTACAAA	ATTTTTTTT	TTTTCGTACT
14651	TGCAAAATAA	AAAACTCACC	AAGGCCATGG	CACCGAGCGT	TGGTTTTCTT
	ACGTTTTATT	TTTTGAGTGG	TTCCGGTACC	GTGGCTCGCA	ACCAAAAGAA
14701	GTATTCCCCT CATAAGGGGA	TAGTATGCGG ATCATACGCC	CGCGCGCGCT	TGTATGAGGA ACATACTCCT	AGGTCCTCCT TCCAGGAGGA
14751	CCCTCCTACG GGGAGGATGC	AGAGTGTGGT TCTCACACCA	GAGCGCGGCG CTCGCGCCGC	CCAGTGGCGG GGTCACCGCC	CGGCGCTGGG
14801	TTCTCCCTTC	GATGCTCCCC	TGGACCCGCC	GTTTGTGCCT	CCGCGGTACC
	AAGAGGGAAG	CTACGAGGGG	ACCTGGGCGG	CAAACACGGA	GGCGCCATGG
14851	TGCGGCCTAC	CGGGGGGAGA	AACAGCATCC	GTTACTCTGA	GTTGGCACCC
	ACGCCGGATG	GCCCCCTCT	TTGTCGTAGG	CAATGAGACT	CAACCGTGGG
14901	CTATTCGACA	CCACCCGTGT	GTACCTGGTG	GACAACAAGT	CAACGGATGT
	GATAAGCTGT	GGTGGGCACA	CATGGACCAC	CTGTTGTTCA	GTTGCCTACA
14951	GGCATCCCTG	AACTACCAGA	ACGACCACAG	CAACTTTCTG	ACCACGGTCA
	CCGTAGGGAC	TTGATGGTCT	TGCTGGTGTC	GTTGAAAGAC	TGGTGCCAGT
15001	TTCAAAACAA	TGACTACAGC	CCGGGGGAGG	CAAGCACACA	GACCATCAAT
	AAGTTTTGTT	ACTGATGTCG	GGCCCCCTCC	GTTCGTGTGT	CTGGTAGTTA
15051	CTTGACGACC GAACTGCTGG	GGTCGCACTG CCAGCGTGAC	GGGCGGCGAC	CTGAAAACCA GACTTTTGGT	TCCTGCATAC AGGACGTATG
15101	CAACATGCCA	AATGTGAACG	AGTTCATGTT	TACCAATAAG	TTTAAGGCGC
	GTTGTACGGT	TTACACTTGC	TCAAGTACAA	ATGGTTATTC	AAATTCCGCG

Figure 26 P

15151	GGGTGATGGT CCCACTACCA	CAGCGCGAAC	CCTACTAAGG GGATGATTCC	ACAATCAGGT TGTTAGTCCA	GGAGCT LA CCTCGACTTT
15201				GGCAACTACT CCGTTGATGA	
15251	CTGGTATCTG	GAATACTTGT	TGCGCTAGCA	GGAGCACTAC CCTCGTGATG	AACTTTCACC
15301				TCGGGGTAAA AGCCCCATTT	
15351	GCGTTGAAGT	CTGACCCCAA	ACTGGGGCAG	ACTGGTCTTG TGACCAGAAC	AGTACGGACC
15401	CCATATATGT	TTGCTTCGGA	AGGTAGGTCT	CATCATTTTG GTAGTAAAAC	GACGGTCCTA
15451	CGCCCCACCT	GAAGTGGGTG	TCGGCGGACT	GCAACTTGTT CGTTGAACAA	CCCGTAGGCG
15501	TTCGCCGTTG	GGAAGGTCCT	CCCGAAATCC	ATCACCTACG TAGTGGATGC	TACTAGACCT
15551		TAAGGGCGTG	ACAACCTACA	CCTGCGGATG	GTCCGCTCGA
15601	ACTTTCTACT	GTGGCTTGTC	CCGCCCCAC	GCGCAGGCGG CGCGTCCGCC	GTCGTTGTCG
15651	TCACCGTCGC	CGCGCCTTCT	CTTGAGGTTG	GCGGCAGCCG	GCCGTTACGT
15701	CGGCCACCTC	CTGTACTTGC	TAGTACGGTA	TCGCGGCGAC AGCGCCGCTG	TGGAAACGGT
15751	GTGCCCGACT	CCTCTTCGCG	CGACTCCGGC	AAGCAGCGGC TTCGTCGCCG	GCTTCGACGG
15801	CGGGGGCGAC	GCGTTGGGCT	CCAGCTCTTC	CCTCAGAAGA GGAGTCTTCT	TTGGCCACTA
15851	GTTTGGGGAC	TETCTCCTGT	CGTTCTTTGC	CAGTTACAAC GTCAATGTTG	GATTATTCGT
		GAAGTGGGTC	ATGGCGTCGA	CCATGGAACG	TATGTTGATG
		TCTGGCCTTA	GGCGAGTACC	TGGGACGAAA	CGTGAGGACT
16001	CGTAACCTGC	GCCTCGGAGC	AGGTCTACTG TCCAGATGAC	GTCGTTGCCA CAGCAACGGT	GACATGATGC CTGTACTACG
16051	AAGACCCCGT TTCTGGGGCA	GACCTTCCGC CTGGAAGGCG	TCCACGCGCC AGGTGCGCGG	AGATCAGCAA TCTAGTCGTT	CTTTCCGGTG GAAAGGCCAC

Figure 26 Q

16101	GTGGGCGCCG CACCCGCGGC	A TGTTGCC T ACAACGG	CGTGCACTCC GCACGTGAGG	AAGAGCTTCT TTCTCGAAGA	TGTTGC GT
16151	GGCCGTCTAC	TCCCAACTCA	TCCGCCAGTT	TACCTCTCTG	ACCCACGTGT
	CCGGCAGATG	AGGGTTGAGT	AGGCGGTCAA	ATGGAGAGAC	TGGGTGCACA
16201	TCAATCGCTT AGTTAGCGAA	TCCCGAGAAC AGGGCTCTTG	CAGATTTTGG GTCTAAAACC	CGCGCCCGCC	AGCCCCCACC TCGGGGGTGG
16251	ATCACCACCG	TCAGTGAAAA	CGTTCCTGCT	CTCACAGATC	ACGGGACGCT
	TAGTGGTGGC	AGTCACTTTT	GCAAGGACGA	GAGTGTCTAG	TGCCCTGCGA
16301	ACCGCTGCGC	AACAGCATCG	GAGGAGTCCA	GCGAGTGACC	ATTACTGACG
	TGGCGACGCG	TTGTCGTAGC	CTCCTCAGGT	CGCTCACTGG	TAATGACTGC
16351	CCAGACGCCG	CACCTGCCCC	TACGTTTACA	AGGCCCTGGG	CATAGTCTCG
	GGTCTGCGGC	GTGGACGGGG	ATGCAAATGT	TCCGGGACCC	GTATCAGAGC
16401	CCGCGCGTCC	TATCGAGCCG	CACTTTTTGA	GCAAGCATGT	CCATCCTTAT
	GGCGCGCAGG	ATAGCTCGGC	GTGAAAAACT	CGTTCGTACA	GGTAGGAATA
16451	ATCGCCCAGC	AATAACACAG	GCTGGGGCCT	GCGCTTCCCA	AGCAAGATGT
	TAGCGGGTCG	TTATTGTGTC	CGACCCCGGA	CGCGAAGGGT	TCGTTCTACA
16501	TTGGCGGGGC	CAAGAAGCGC	TCCGACCAAC	ACCCAGTGCG	CCTGCGCGGG
	AACCGCCCCG	GTTCTTCGCG	AGGCTGGTTG	TGGGTCACGC	GCACGCGCCC
16551	CACTACCGCG	CGCCCTGGGG	CGCGCACAAA	CGCGGCCGCA	CTGGGCGCAC
	GTGATGGCGC	GCGGGACCCC	GCGCGTGTTT	GCGCCGGCGT	GACCCGCGTG
16601	CACCGTCGAT	GACGCCATCG	ACGCGGTGGT	GGAGGAGGCG	CGCAACTACA
	GTGGCAGCTA	CTGCGGTAGC	TGCGCCACCA	CCTCCTCCGC	GCGTTGATGT
16651	CGCCCACGCC	GCCACCAGTG CGGTGGTCAC	TCCACAGTGG AGGTGTCACC	ACGCGGCCAT TGCGCCGGTA	TCAGACCGTG AGTCTGGCAC
16701	GTGCGCGGAG	CCCGGCGCTA	TGCTAAAATG	AAGAGACGGC	GGAGGCGCGT
	CACGCGCCTC	GGGCCGCGAT	ACGATTTTAC	TTCTCTGCCG	CCTCCGCGCA
16751	AGCACGTCGC TCGTGCAGCG	CACCGCCGCC GTGGCGGCGG	GACCCGGCAC CTGGGCCGTG	TGCCGCCCAA ACGGCGGTT	CCCCCCCCC
16801	CGGCCCTGCT	TAACCGCGCA	CGTCGCACCG	GCCGACGGGC	GGCCATGCGG
	GCCGGGACGA	ATTGGCGCGT	GCAGCGTGGC	CGGCTGCCCG	CCGGTACGCC
16851	GCCGCTCGAA	GGCTGGCCGC	GGGTATTGTC	ACTGTGCCCC	CCAGGTCCAG
	CGGCGAGCTT	CCGACCGGCG	CCCATAACAG	TGACACGGGG	GGTCCAGGTC
16901	GCGACGAGCG	GCCGCCGCAG	CAGCCGCGGC	CATTAGTGCT	ATGACTCAGG
	CGCTGCTCGC	CGCCGCCGTC	GTCGGCGCCG	GTAATCACGA	TACTGAGTCC
16951	GTCGCAGGGG CAGCGTCCCC	CAACGTGTAT GTTGCACATA	TGGGTGCGCG ACCCACGCGC	ACTCGGTTAG TGAGCCAATC	CGGCCTGCGC
17001	GTGCCCGTGC CACGGGCACG	GCACCCGCCC CGTGGGCGGG	CCCGCGCAAC	TAGATTGCAA ATCTAACGTT	GAAAAAACTA CTTTTTTGAT



17051	CTTAGACTCG GAATCTGAGC	T GTTGTA ATGACAACAT	TGTATCCAGC ACATAGGTCG	ccecceccec	GCGTTGC+TC
17101		GCGCAAAATC CGCGTTTTAG			
17151		GCCCCCGAA CGGGGGGCTT			
17201		GTCAAAAAGA CAGTTTTTCT			
17251		ACTGCTGCAC TGACGACGTG			
17301		GCGTAAAACG CGCATTTTGC			
17351		GAGCGCTCCA CTCGCGAGGT			
17401		CGAGGACCTG GCTCCTGGAC			
17451		GAAAGCGGCA CTTTCGCCGT			
17501		ACACCTAGCC TGTGGATCGG			
17551		ACCGTCCGAA TGGCAGGCTT			
.17601		CCACCGTGCA GGTGGCACGT			
17651		GAAAAAATGA CTTTTTTACT			
17701		AATCAAGCAG TTAGTTCGTC			
17751	GACGTTCAGA CTGCAAGTCT	TACCCACTAC ATGGGTGATG	CAGTAGCACC GTCATCGTGG	AGTATTGCCA TCATAACGGT	CCGCCACAGA GGCGGTGTCT
17801	GGGCATGGAG CCCGTACCTC	ACACAAACGT TGTGTTTGCA			
17851	CGGTGCAGGC GCCACGTCCG	GGTCGCTGCG CCAGCGACGC	GCCGCGTCCA CGGCGCAGGT	AGACCTCTAC TCTGGAGATG	GGAGGTGCAA CCTCCACGTT
17901	ACGGACCCGT TGCCTGGGCA	GGATGTTTCG CCTACAAAGC	CGTTTCAGCC GCAAAGTCGG	GGGGCGGGG CCCGGGGGGCC	CGCGCCGTTC
17951	GAGGAAGTAC CTCCTTCATG				GCCCTACATC CGGGATGTAG

Tigure 265

18001	CTTCCATTGC GAAGGTAACG	GCCTACCCCC	GGCTATCGTG CCGATAGCAC	GCTACACCTAL CGATGTGGAT	CCCCCCCTT GCCGCCTT
18051	AGACGAGCAA	CTACCCGACG	CCGAACCACC	ACTGGAACCC	922929292
	TCTGCTCGTT	GATGGGCTGC	GGCTTGGTGG	TGACCTTGGG	00092929292
18101	TCGCCGTCGC	CAGCCCGTGC	TGGCCCCGAT	TTCCGTGCGC	AGGGTGGCTC
	AGCGGCAGCG	GTCGGGCACG	ACCGGGGCTA	AAGGCACGCG	TCCCACCGAG
18151	GCGAAGGAGG	CAGGACCCTG	GTGCTGCCAA	CAGCGCGCTA	CCACCCCAGC
	CGCTTCCTCC	GTCCTGGGAC	CACGACGGTT	GTCGCGCGAT	GGTGGGGTCG
18201	ATCGTTTAAA	AGCCGGTCTT	TGTGGTTCTT	GCAGATATGG	CCCTCACCTG
	TAGCAAATTT	TCGGCCAGAA	ACACCAAGAA	CGTCTATACC	GGGAGTGGAC
18251	CCGCCTCCGT	TTCCCGGTGC	CGGGATTCCG	AGGAAGAATG	CACCGTAGGA
	GGCGGAGGCA	AAGGGCCACG	GCCCTAAGGC	TCCTTCTTAC	GTGGCATCCT
18301	GGGGCATGGC	CGGCCACGGC	CTGACGGGCG	GCATGCGTCG	TGCGCACCAC
	CCCCGTACCG	GCCGGTGCCG	GACTGCCCGC	CGTACGCAGC	ACGCGTGGTG
18351	CGCCGCCGC	GCGCGTCGCA CGCGCAGCGT	CCGTCGCATG GGCAGCGTAC	CGCGGCGGTA GCGCCGCCAT	TCCTGCCCCT AGGACGGGGA
18401	CCTTATTCCA GGAATAAGGT	CTGATCGCCG GACTAGCGGC	CGGCGATTGG GCCGCTAACC	CGCCGTGCCC	GGAATTGCAT CCTTAACGTA
18451	CCGTGGCCTT	GCAGGCGCAG	AGACACTGAT	TAAAAACAAG	TTGCATGTGG
	GGCACCGGAA	CGTCCGCGTC	TCTGTGACTA	ATTTTTGTTC	AACGTACACC
18501	AAAAATCAAA	ATAAAAAGTC	TGGACTCTCA	CGCTCGCTTG	GTCCTGTAAC
	TTTTTAGTTT	TATTTTTCAG	ACCTGAGAGT	GCGAGCGAAC	CAGGACATTG
18551	TATTITGTAG	AATGGAAGAC	ATCAACTTTG	CGTCTCTGGC	CCCGCGACAC
	ATAAAACATC	TTACCTTCTG	TAGTTGAAAC	GCAGAGACCG	GGGCGCTGTG
18601	GGCTCGCGCC	CGTTCATGGG	AAACTGGCAA	GATATCGGCA	CCAGCAATAT
	CCGAGCGCGG	GCAAGTACCC	TTTGACCGTT	CTATAGCCGT	GGTCGTTATA
18651	GAGCGGTGGC CTCGCCACCG	GCCTTCAGCT CGGAAGTCGA	GGGGCTCGCT	GTGGAGCGGC CACCTCGCCG	TTAAAAATT AATTTTTAA
18701	TCGGTTCCAC	CGTTAAGAAC	TATGGCAGGA	AGGCCTGGAA	CAGCAGCACA
	AGCCAAGGTG	GCAATTCTTG	ATACCGTCGT	TCCGGACCTT	GTCGTCGTGT
18751	GGCCAGATGC	TGAGGGATAA	GTTGAAAGAG	CAAAATTTCC	AACAAAAGGT
	CCGGTCTACG	ACTCCCTATT	CAACTTTCTC	GTTTTAAAGG	TTGTTTTCCA
18801	GGTAGATGGC	CTGGCCTCTG	GCATTAGCGG	GCTGGTGGAC	CTGGCCAACC
	CCATCTACCG	GACCGGAGAC	CGTAATCGCC	CCACCACCTG	GACCGGTTGG
18851	AGGCAGTGCA	AAATAAGATT	AACAGTAAGC	TTGATCCCCG	CCCTCCCGTA
	TCCGTCACGT	TTTATTCTAA	TTGTCATTCG	AACTAGGGGC	GGGAGGGCAT
18901	GAGGAGCCTC	CACCGGCCGT	GGAGACAGTG	TCTCCAGAGG	GGCGTGGCGA
	CTCCTCGGAG	GTGGCCGGCA	CCTCTGTCAC	AGAGGTCTCC	CCGCACCGCT

Figure 26T

18951	AAAGCGTCCG TTTCGCAGGC	CCGACA GCGGGCTGT	GGGAAGAAAC CCCTTCTTTG	TCTGGTGACG AGACCACTGC	CAAATA G
19001		GTACGAGGAG CATGCTCCTC			
19051		CCATGGCTAC GGTACCGATG			
19101		CCTCCCCCG GGAGGGGGC			
19151		CGTTGTTGTA GCAACAACAT			
19201		GTCCGCGATC CAGGCGCTAG			
19251		AACAGCATCG TTGTCGTAGC			
19301		CTGATAGCTA GACTATCGAT			
19351		CAGAGGAGCT GTCTCCTCGA			
19401		TTCGATGATG AAGCTACTAC			
19451		CGGAGTACCT GCCTCATGGA			
19501		TACTTCAGCC ATGAAGTCGG			
19551		CGACGTGACC GCTGCACTGG			
19601		TGGACCGTGA ACCTGGCACT			
19651		GTGGGTGATA CACCCACTAT			
19701	TTGACATCCG AACTGTAGGC	CGGCGTGCTG GCCGCACGAC	GACAGGGGCC CTGTCCCCGG	CTACTTTTAA GATGAAAATT	GCCCTACTCT CGGGATGAGA
19751	GGCACTGCCT CCGTGACGGA	ACAACGCCCT TGTTGCGGGA			
19801	ATGGGATGAA TACCCTACTT	GCTGCTACTG CGACGATGAC	CTCTTGAAAT GAGAACTTTA	AAACCTAGAA TTTGGATCTT	GAAGAGGACG CTTCTCCTGC
19851	ATGACAACGA TACTGTTGCT	AGACGAAGTA TCTGCTTCAT	GACGAGCAAG CTGCTCGTTC	CTGAGCAGCA GACTCGTCGT	AAAAACTCAC TTTTTGAGTG

Figure 26 U

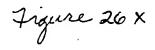
		TCCGCGGAAT	AAGACCATAT	TTATAATGTT	TCCTCCATA
•		CAGCTTCCAG	TTTGTGGATT	TATACGGCTA	TTTTGTAAAG
20001		AGTTTATCCT	CTTAGAGTCA	CCATGCTTTG	TCTTTAATTA
20051	GTACGTCGAC	CCTCTCAGGA	TTTTTTCTGA	ACCCCAATGA TGGGGTTACT	TTGGTACAAT
20101	GCCAAGTATA	CGTTTTGGGT	GTTTACTTTT	TGGAGGGCAA ACCTCCCGTT	CCGTAAGAAC
20151	ATTTCGTTGT	TTTACCTTTC	GATCTTTCAG	AAGTGGAAAT TTCACCTTTA	CGTTAAAAAG
20201	AGTTGATGAC	TCCGTCGGCG	TCCGTTACCA	GATAACTTGA CTATTGAACT	GAGGATTTCA
20251	CCATAACATG	TCACTTCTAC	ATCTATATCT		TGAGTATAAA
20301	GAATGTACGG	GTGATAATTC	CTTCCATTGA	CACGAGAACT GTGCTCTTGA	TTACCCGGTT
20351	GTTAGATACG	GGTTGTCCGG	ATTAATGTAA	GCTTTTAGGG CGAAAATCCC	TGITAAAATA
20401	ACCAGATTAC	ATAATGTTGT	CGTGCCCATT	TATGGGTGTT ATACCCACAA	GACCGCCCGG
20451	TTCGTAGCGT	CAACTTACGA	CAACATCTAA	TGCAAGACAG ACGTTCTGTC	TTTGTGTCTC
20501	GAAAGTATGG	TCGAAAACGA	ACTAAGGTAA	GGTGATAGAA	GGTCCATGAA
20551	AAGATACACC	TTAGTCCGAC	AACTGTCGAT	TGATCCAGAT ACTAGGTCTA	CAATCTTAAT
20601	AACTTTTAGT	ACCTTGACTT	CTACTTGAAG	CAAATTACTG GTTTAATGAC	GAAAGGTGAC
	CCTCCACACT	AATTATGTCT	CTGAGAATGG	TTCCATTTTG	CTAAAACAGG GATTTTGTCC
	AGTCCTTTTA	CCTACCCTTT	TTCTACGATG	TCTTAAAAGT	GATAAAAATG CTATTTTTAC
	TTTATTCTCA	ACCTTTATTA	AAACGGTACC	TTTAGTTAGA	AAATGCCAAC TTTACGGTTG
20801	CTGTGGAGAA GACACCTCTT	ATTTCCTGTA TAAAGGACAT	CTCCAACATA GAGGTTGTAT	GCGCTGTATT CGCGACATAA	TGCCCGACAA ACGGGCTGTT

Figure 26 V

20851	GCTAAAGTAC CGATTTCATG	ACCTTCCA TGGAAGGT	ACGTAAAAAT TGCATTTTTA	TTCTGATÄÄČ AAGACTATTG	TOTAL CONTROL OF THE
20901	ACGACTACAT TGCTGATGTA	GAACAAGCGA CTTGTTCGCT	GTGGTGGCTC CACCACCGAG	CCGGGCTAGT GGCCCGATCA	GGACTGCTAC CCTGACGATG
20951				TATATGGACA ATATACCTGT	
21001				CTACCGCTCA GATGGCGAGT	
21051	CGTTACCAGC	GATACACGGG	AAGGTGTAGG	AGGTGCCTCA TCCACGGAGT	CTTCAAGAAA
21101	CGGTAATTTT	TGGAGGAAGA	GGACGGCCCG	TCATACACCT AGTATGTGGA	TGCTCACCTT
21151		CTACAATTGT	ACCAAGACGT	CTCGAGGGAT	CCTTTACTGG
21201	ATTCCCAACT	GCCTCGGTCG	TAATTCAAAC	ATAGCATTTG TATCGTAAAC	GGAAATGCGG
21251	TGGAAGAAGG	GGTACCGGGT	GTTGTGGCGG	TCCACGCTTG AGGTGCGAAC	TCCGGTACGA
21301	ATCTTTGCTG	TGGTTGCTGG	TCAGGAAATT	CGACTATCTC GCTGATAGAG	AGGCGGCGGT
21351		GGGATATGGG	CGGTTGCGAT	GGTTGCACGG	GTATAGGTAG
21401	GGGAGGGCGT	TGACCCGCCG	AAAGGCGCCG	TGGGCCTTCA ACCCGGAAGT	GCGCGGAATT
21451	GACTAAGGAA CTGATTCCTT	TGGGGTAGTG	ACCCGAGCCC	GATGCTGGGA	ATAATGTGGA
21501	TGAGACCGAG	ATATGGGATG	GATCTACCTT	CCTTTTACCT GGAAAATGGA	GTTGGTGTGG
21551	AAATTCTTCC	ACCGGTAATG	GAAACTGAGA	TCTGTCAGCT AGACAGTCGA	CCGGACCGTT
		GAATGGGGGT	TGCTCAAACT	TTAATTCGCG	AGTCAACTGC
		GTTGCAACGG	GTCACATTGT	ACTGGTTTCT	GACCAAGGAC
		ATCGATTGAT	ATTGTAACCG	ATGGTCCCGA	AGATATAGGG
21751	AGAGAGCTAC TCTCTCGATG	AAGGACCGCA TTCCTGGCGT	TGTACTCCTT ACATGAGGAA	CTTTAGAAAC GAAATCTTTG	TTCCAGCCCA AAGGTCGGGT

Figure 26 W

21801	TCACCCCTCA	ССТССТССАТ	CATACTAAAT	ACAAGGACTA	-ECAACAGETG
21601	ACTCGGCAGT	CCACCTA	CTATGATTTA	TGTTCCTGAT	GGTTG1 .C
21851	GGCATCCTAC	ACCAACACAA	CAACTCTGGA	TTTGTTGGCT	ACCTTGCCCC
				AAACAACCGA	
21901	CACCATGCGC	GAAGGACAGG	CCTACCCTGC	TAACTTCCCC	TATCCGCTTA
				ATTGAAGGGG	
21951	TAGGCAAGAC	CGCAGTTGAC	ACCATTACCC	AGAAAAAGTT	TCTTTGCGAT
				TCTTTTTCAA	
22001	CGCACCCTTT	GGCGCATCCC	ATTCTCCAGT	AACTTTATGT	CCATGGGCGC
				TTGAAATACA	
22051	ACTCACAGAC	CTGGGCCAAA	ACCTTCTCTA	CGCCAACTCC	GCCCACGCGC
	•			GCGGTTGAGG	
22101	TAGACATGAC	TTTTGAGGTG	GATCCCATGG	ACGAGCCCAC	CCTTCTTTAT
				TGCTCGGGTG	
22151	GTTTTGTTTG	AAGTCTTTGA	CGTGGTCCGT	GTGCACCAGC	CGCACCGCGG
				CACGTGGTCG	
22201	CGTCATCGAA	ACCGTGTACC	TGCGCACGCC	CTTCTCGGCC	GGCAACGCCA
				GAAGAGCCGG	
22251	CAACATAAAG	AAGCAAGCAA	CATCAACAAC	AGCTGCCGCC	MACCCCACCT
				TCGACGGCGG	
22301	GTGAGCAGGA	ACTGAAAGCC	ATIGICAAAG	ATCTTGGTTG TAGAACCAAC	ACCCCCTATA
				GGCTTTGTTT	
22351	TTTTTGGGCA	CCTATGACAA	CCCCN NACCT	CCGAAACAAA	CACCTCTCTT
				TCGCGAGACT	
22401	GCTCGCCTGC	GCCATAGTCA	MATACGGCCGGCC	AGCGCTCTGA	CCCCCCCATG
	CGAGCGGACG	CGGIAICAGI	INIGCEGGCC	AGCGC1C1G.	500000000000000000000000000000000000000
22451	ACTGGATGGC	CTTTGCCTGG	AACCCGCACT	CAAAAACATG	CTACCTCTTT
22431	TGACCTACCG	GAAACGGACC	TTGGGCGTGA	GTTTTTGTAC	GATGGAGAAA
22501	GAGCCCTTTG	GCTTTTCTGA	CCAGCGACTC	AAGCAGGTTT	ACCAGTTTGA
	CTCGGGAAAC	CGAAAAGACT	GGTCGCTGAG	TTCGTCCAAA	TGGTCAAACT
22551	GTACGAGTCA	CTCCTGCGCC	GTAGCGCCAT	TGCTTCTTCC	CCCGACCGCT
	CATGCTCAGT	GAGGACGCGG	CATCGCGGTA	ACGAAGAAGG	GGGCTGGCGA
22601	GTATAACGCT	GGAAAAGTCC	ACCCAAAGCG	TACAGGGGCC	CAACTCGGCC
			•		GTTGAGCCGG
22651	GCCTGTGGAC	TATTCTGCTG	CATGTTTCTC	CACGCCTTTG	CCAACTGGCC
					GGTTGACCGG
22701	CCAAACTCCC	ATGGATCACA	ACCCCACCAT	GAACCTTATT	ACCGGGGTAC
	GGTTTGAGGG	TACCTAGTGT	TGGGGTGGTA	CTTGGAATAA	TGGCCCCATG



22751	CCAACTCCAT GGTTGAGGTA	GCTCAACAGT CTTGTCA	CCCCAGGTAC GGGGTCCATG	AGCCCACQGA TCGGGTGGGA	CCAGC CAR
22801			CCTGGAGCGC GGACCTCGCG		
22851			GCGCCACTTC CGCGGTGAAG		
22901			GACACTTTCA CTGTGAAAGT		
22951			ATTTACCCCC TAAATGGGGG		
23001			GCCGCGCATC CGGCGCGTAG		
23051			TTAGTGCTCC AATCACGAGG		
23101			GTTTTCACTC CAAAAGTGAG		
23151			GCGCCGATAT CGCGGCTATA		
23201	CTCCGCCCTG GAGGCGGGAC	CGCGCGCGAG GCGCGCGCTC	TTGCGATACA AACGCTATGT	CAGGGTTGCA GTCCCAACGT	GCACTGGAAC CGTGACCTTG
23251			CACGCTGGCC GTGCGACCGG		
23301			CCGCGTTGCT GGCGCAACGA		
23351			AAGGGCGCGT TTCCCGCGCA	•	
23401			AAGGTGACCG TTCCACTGGC		
23451			CCTTGATCTG GGAACTAGAC		
23501	TTGCGCCTTC AACGCGGAAG	AGAGAAGAAC TCTCTTCTTG	ATGCCGCAAG TACGGCGTTC	ACTTGCCGGA TGAACGGCCT	AAACTGATTG TTTGACTAAC
23551	GCCGGACAGG CGGCCTGTCC		CACGCAGCAC GTGCGTCGTG		
23601	CTGCACCACA GACGTGGTGT		ACCGGTTCTT TGGCCAAGAA		
23651	ACTGCTCCTT TGACGAGGAA		TGCCCGTTTT ACGGGCAAAA		

Figure 26 Y

23701	ATCACGTGCT TAGTGCACGA	CC PATTTAT GGAATAAATA	CATAATGCTT GTATTACGAA	CCGTGTAGAC GGCACATCTG	ACTTAA CC TGAATTCGAG
23751	GCCTTCGATC CGGAAGCTAG	TCAGCGCAGC AGTCGCGTCG	GGTGCAGCCA CCACGTCGGT	CAACGCGCAG GTTGCGCGTC	CCCGTGGGCT GGGCACCCGA
23801	GCACTACGAA	CATCCAGTGG	TCTGCAAACG AGACGTTTGC	TGACGTCCAT	GCGGACGTCC
23851	AATCGCCCCA TTAGCGGGGT	TCATCGTCAC AGTAGCAGTG	AAAGGTCTTG TTTCCAGAAC	TTGCTGGTGA AACGACCACT	AGGTCAGCTG TCCAGTCGAC
23901	CAACCCGCGG GTTGGGCGCC	TGCTCCTCGT ACGAGGAGCA	TCAGCCAGGT AGTCGGTCCA	CTTGCATACG GAACGTATGC	GCCGCCAGAG CGGCGGTCTC
23951	GAAGGTGAAC	CAGTCCGTCA	AGTTTGAAGT TCAAACTTCA	AGCGGAAATC	TAGCAATAGG
24001	TGCACCATGA	ACAGGTAGTC	CGCGCGCGCA GCGCGCGCGT	CGGAGGTACG	GGAAGAGGGT
24051	GCGTCTGTGC	TAGCCGTGTG	TCAGCGGGTT AGTCGCCCAA	GTAGTGGCAT	TAAAGTGAAA
24101	GGCGAAGCGA	CCCGAGAAGG	TCTTCCTCTT AGAAGGAGAA	CGCAGGCGTA	TGGTGCGCGG
24151	TGACCCAGCA	GAAGTAAGTC	CCGCCGCACT GGCGGCGTGA	CACGCGAATG	GAGGAAACGG
24201	TACGAACTAA	TCGTGGCCAC	CCAACGACTT	TGGGTGGTAA	
24251	GTAGAAGAGA	AAGAAGGAGC	CTGTCCACGA GACAGGTGCT	AATGGAGACC	ACTACCGCCC
24301	GCGAGCCCGA	ACCCTCTTCC	GCGCTTCTTT	AAGAAGAACC	CGCGTTACCG
24351	GTTTAGGCGG	CGGCTCCAGC	TACCGGCGCC	CGACCCACAC	CGCGGCACCA GCGCCGTGGT
24401	CGCGCAGAAC	ACTACTCAGA	AGGAGCAGGA	GCCTGAGCTA	ACGCCGCCTC TGCGGCGGAG
	TAGGCGAAAA	AACCCCCGCG	GGCCCCTCCG	CCGCCGCTGC	GGGACGGGGA CCCTGCCCCT
	GCTGTGCAGG	AGGTACCAAC	CCCCTGCAGC	GCGGCGTGGC	CGTCCGCGCT GCAGGCGCGA
	GCCCCACCA	AAGCGCGACG	AGGAGAAGGG	CTGACCGGTA	TTCCTTCTCC AAGGAAGAGG
24601	TATAGGCAGA ATATCCGTCT	AAAAGATCAT TTTTCTAGTA	GGAGTCAGTC CCTCAGTCAG	GAGAAGAAGG CTCTTCTTCC	ACAGCCTAAC TGTCGGATTG

Figure 262

24651	CGCCCCCTCT GCGGGGGAGA	TCGCCA CTCAAGCGGT	CCACCGCCTC GGTGGCGGAG	CACCGATGCC GTGGCTACGG	GCCAAC CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
24701				TTGAGGAGGA AACTCCTCCT	
24751				GACGACGAGG CTGCTGCTCC	
24801				CAACGCAGAG GTTGCGTCTC	
24851				GCGACTACCT CGCTGATGGA	
24901		-		CAGTGCGCCA GTCACGCGGT	
24951				CGCCATAGCG GCGGTATCGC	
25001				GCGTACCCCC CGCATGGGGG	
25051				CTCAACTTCT GAGTTGAAGA	
25101				CATCTTTTC GTAGAAAAAG	
25151				GCCGAGCGGA CGGCTCGCCT	
25201				ATCGCCTCGC TAGCGGAGCG	
25251				CGAGAAGCGC GCTCTTCGCG	
25301	GAGACGTTGT	CCTTTTGTCG	CTTTTACTTT	GTCACTCTGG CAGTGAGACC	TCACAACCAC
25351	CTTGAGCTCC	CACTGTTGCG	CGCGGATCGG	GTACTAAAAC CATGATTTTG	CGTCGTAGCT
		AAACGGATGG	GCCGTGAATT	GGATGGGGG	TTCCAGTACT
		CTCACTCGAC	TAGCACGCGG	CACGCGTCGG	GGACCTCTCC
	•	ACGTTCTTGT	TTGTCTCCTC	CCGGATGGGC	GTCAACCGCT
25551	CGAGCAGCTA GCTCGTCGAT			CGAGCCTGCC GCTCGGACGG	

7 igure 2 E AA

25601	AGCGACGCAA	AATGATG	GCCGCAGTGC	TCGTTACCGT	GGAGCT G
	TCGCTGCGTT	TGATTACTAC	CGCCTCACG	AGCAATGGCA	CCTCGAACTC
25651	TGCATGCAGC	GGTTCTTTGC	TGACCCGGAG	ATGCAGCGCA	AGCTAGAGGA
	ACGTACGTCG	CCAAGAAACG'	ACTGGGCCTC	TACGTCGCGT	TCGATCTCCT
25701	AACATTGCAC	TACACCTTTC	GACAGGGCTA	CGTACGCCAG	GCCTGCAAGA
	TTGTAACGTG	ATGTGGAAAG	CTGTCCCGAT	GCATGCGGTC	CGGACGTTCT
25751	TCTCCAACGT	GGAGCTCTGC	AACCTGGTCT	CCTACCTTGG	AATTTTGCAC
	AGAGGTTGCA	CCTCGAGACG	TTGGACCAGA	GGATGGAACC	TTAAAACGTG
25801	GAAAACCGCC	TTGGGCAAAA	CGTGCTTCAT	TCCACGCTCA	AGGGCGAGGC
	CTTTTGGCGG	AACCCGTTTT	GCACGAAGTA	AGGTGCGAGT	TCCCGCTCCG
25851		TACGTCCGCG ATGCAGGCGC			
25901		CATGGGCGTT GTACCCGCAA			
25951		AGAAACTGCT TCTTTGACGA			
26001	CTTCAACGAG	CGCTCCGTGG	CCGCGCACCT	GGCGGACATC	ATTTTCCCCG
	GAAGTTGCTC	GCGAGGCACC	GGCGCGTGGA	CCGCCTGTAG	TAAAAGGGGC
26051	AACGCCTGCT	TAAAACCCTG	CAACAGGGTC	TGCCAGACTT	CACCAGTCAA
	TTGCGGACGA	ATTTTGGGAC	GTTGTCCCAG	ACGGTCTGAA	GTGGTCAGTT
26101		AGAACTTTAG TCTTGAAATC			
26151	GCCCGCCACC	TGCTGTGCAC	TTCCTAGCGA	CTTTGTGCCC	ATTAAGTACC
	CGGGCGGTGG	ACGACACGTG	AAGGATCGCT	GAAACACGGG	TAATTCATGG
26201		TCCGCCGCTT AGGCGGCGAA			
26251	AACTACCTTG	CCTACCACTC	TGACATAATG	GAAGACGTGA	GCGGTGACGG
	TTGATGGAAC	GGATGGTGAG	ACTGTATTAC	CTTCTGCACT	CGCCACTGCC
26301	TCTACTGGAG AGATGACCTC	TGTCACTGTC ACAGTGACAG	GCTGCAACCT CGACGTTGGA	ATGCACCCCG TACGTGGGGC	CACCGCTCCC
26351	TGGTTTGCAA	TTCGCAGCTG	CTTAACGAAA	GTCAAATTAT	CGGTACCTTT
	ACCAAACGTT	AAGCGTCGAC	GAATTGCTTT	CAGTTTAATA	GCCATGGAAA
26401	GAGCTGCAGG	GTCCCTCGCC	TGACGAAAAG	TCCGCGGCTC	CGGGGTTGAA
	CTCGACGTCC	CAGGGAGCGG	ACTGCTTTTC	AGGCGCCGAG	GCCCCAACTT
26451	ACTCACTCCG	GGGCTGTGGA	CGTCGGCTTA	CCTTCGCAAA	TTTGTACCTG
	TGAGTGAGGC	CCCGACACCT	GCAGCCGAAT	GGAAGCGTTT	AAACATGGAC
26501	AGGACTACCA	CGCCCACGAG	ATTAGGTTCT	ACGAAGACCA	ATCCCGCCCG
	TCCTGATGGT	GCGGGTGCTC	TAATCCAAGA	TGCTTCTGGT	TAGGGCGGGC

Figure 26 AB

26551				ACCCAGGGCC TGGGTCCCGG	
26601				AGAGTTTCTG TCTCAAAGAC	
26651				GCGAGGAGCT CGCTCCTCGA	
26701				CCGCGGGCCC	
26751	GGATGGCACC CCTACCGTGG	CAAAAAGAAG GTTTTTCTTC	CTGCAGCTGC GACGTCGACG	CGCCGCCACC GCGGCGGTGG	CACGGACGAG GTGCCTGCTC
26801	GAGGAATACT CTCCTTATGA	GGGACAGTCA CCCTGTCAGT	GGCAGAGGAG CCGTCTCCTC	GTTTTGGACG CAAAACCTGC	AGGAGGAGGA TCCTCCTCCT
26851				CGAGGAAGCT GCTCCTTCGA	
26901				CGGTCGCATT GCCAGCGTAA	
26951				ATGGCTACAA TACCGATGTT	
27001				ACCCAACCGT TGGGTTGGCA	
27051	CCACTGGAAC GGTGACCTTG	CAGGGCCGGT GTCCCGGCCA	AAGTCCAAGC TTCAGGTTCG	AGCCGCCGCC TCGGCGGCGG	GTTAGCCCAA CAATCGGGTT
27101	GAGCAACAAC CTCGTTGTTG	AGCGCCAAGG TCGCGGTTCC	CTACCGCTCA GATGGCGAGT	TGGCGCGGGC ACCGCGCCCG	ACAAGAACGC TGTTCTTGCG
27151				CAACATCTCC GTTGTAGAGG	
27201				TCCCCCGTAA AGGGGGCATT	
27251	TACTACCGTC ATGATGGCAG	ATCTCTACAG TAGAGATGTC	CCCATACTGC GGGTATGACG	ACCGGCGGCA TGGCCGCCGT	GCGGCAGCAA CGCCGTCGTT
27301	CAGCAGCGGC GTCGTCGCCG	CACACAGAAG GTGTGTCTTC	CAAAGGCGAC GTTTCCGCTG	CCGATAGCAA GCCTATCGTT	GACTCTGACA CTGAGACTGT
27351	AAGCCCAAGA TTCGGGTTCT	AATCCACAGC TTAGGTGTCG	GGCGGCAGCA CCGCCGTCGT	GCAGGAGGAG CGTCCTCCTC	GAGCGCTGCG CTCGCGACGC
27401	TCTGGCGCCC AGACCGCGGG				AACAGGATTT TTGTCCTAAA
27451	TTCCCACTCT AAGGGTGAGA	GTATGCTATA CATACGATAT	TTTCAACAGA AAAGTTGTCT	GCAGGGGCCA CGTCCCCGGT	AGAACAAGAG TCTTGTTCTC

Figure 26 AC

27501	CTGAAAATAA	A CAGGTC	TCTGCGATCC	CTCACCCGCA	GCTGCC TA
	GACTTTTATT	TTTTGTCCAG	AGACGCTAGG	GAGTGGGCGT	CGACGGACAT
27551	TCACAAAAGC	GAAGATCAGC	TTCGGCGCAC	GCTGGAAGAC	GCGGAGGCTC
	AGTGTTTTCG	CTTCTAGTCG	AAGCCGCGTG	CGACCTTCTG	CGCCTCCGAG
27601	TCTTCAGTAA	ATACTGCGCG	CTGACTCTTA	AGGACTAGTT	TCGCGCCCTT
	AGAAGTCATT	TATGACGCGC	GACTGAGAAT	TCCTGATCAA	AGCGCGGGAA
27651	TCTCAAATTT	AAGCGCGAAA	ACTACGTCAT	CTCCAGCGGC	CACACCGGC
	AGAGTTTAAA	TTCGCGCTTT	TGATGCAGTA	GAGGTCGCCG	GTGTGGGCCG
27701	GCCAGCACCT	GTTGTCAGCG	CCATTATGAG	CAAGGAAATT	CCCACGCCCT
	CGGTCGTGGA	CAACAGTCGC	GGTAATACTC	GTTCCTTTAA	GGGTGCGGGA
27751	ACATGTGGAG	TTACCAGCCA	CAAATGGGAC	TTGCGGCTGG	AGCTGCCCAA
	TGTACACCTC	AATGGTCGGT	GTTTACCCTG	AACGCCGACC	TCGACGGGTT
27801	GACTACTCAA	CCCGAATAAA	CTACATGAGC	GCGGGACCCC	ACATGATATC
	CTGATGAGTT	GGGCTTATTT	GATGTACTCG	CGCCCTGGGG	TGTACTATAG
27851	CCGGGTCAAC	GGAATACGCG	CCCACCGAAA	CCGAATTCTC	CTGGAACAGG
	GGCCCAGTTG	CCTTATGCGC	GGGTGGCTTT	GGCTTAAGAG	GACCTTGTCC
27901	CGGCTATTAC	CACCACACCT	CGTAATAACC	TTAATCCCCG	TAGTTGGCCC
	GCCGATAATG	GTGGTGTGGA	GCATTATTGG	AATTAGGGGC	ATCAACCGGG
27951	GCTGCCCTGG	TGTACCAGGA	AAGTCCCGCT	CCCACCACTG	TGGTACTTCC
	CGACGGGACC	ACATGGTCCT	TTCAGGGCGA	GGGTGGTGAC	ACCATGAAGG
28001	CAGAGACGCC	CAGGCCGAAG	TTCAGATGAC	TAACTCAGGG	GCGCAGCTTG
	GTCTCTGCGG	GTCCGGCTTC	AAGTCTACTG	ATTGAGTCCC	CGCGTCGAAC
28051	CGGGCGGCTT GCCCGCCGAA	TCGTCACAGG AGCAGTGTCC	GTGCGGTCGC CACGCCAGCG	CCGGGCAGGG	TATAACTCAC ATATTGAGTG
28101	CTGACAATCA	GAGGGCGAGG	TATTCAGCTC	AACGACGAGT	CGGTGAGCTC
	GACTGTTAGT	CTCCCGCTCC	ATAAGTCGAG	TTGCTGCTCA	GCCACTCGAG
28151	CTCGCTTGGT GAGCGAACCA	CTCCGTCCGG GAGGCAGGCC	ACGGGACATT TGCCCTGTAA	TCAGATCGGC AGTCTAGCCG	CCGCGGCCGGCC
28201	GCTCTTCATT	CACGCCTCGT	CAGGCAATCC	TAACTCTGCA	GACCTCGTCC
	CGAGAAGTAA	GTGCGGAGCA	GTCCGTTAGG	ATTGAGACGT	CTGGAGCAGG
28251	TCTGAGCCGC	GCTCTGGAGG	CATTGGAACT	CTGCAATTTA	TTGAGGAGTT
	AGACTCGGCG	CGAGACCTCC	GTAACCTTGA	GACGTTAAAT	AACTCCTCAA
28301	TGTGCCATCG ACACGGTAGC	GTCTACTTTA CAGATGAAAT	ACCCCTTCTC TGGGGAAGAG	GGGACCTCCC	GGCCACTATC CCGGTGATAG
28351	CGGATCAATT GCCTAGTTAA	TATTCCTAAC ATAAGGATTG	TTTGACGCGG AAACTGCGCC	TAAAGGACTC ATTTCCTGAG	GCCGCACGGC
28401	TACGACTGAA	TGTTAAGTGG	AGAGGCAGAG	CAACTGCGCC	TGAAACACCT
	ATGCTGACTT	ACAATTCACC	TCTCCGTCTC	GTTGACGCGG	ACTTTGTGGA

Figure 26 AD

28451			AGTGCTTTGC TCACGAAACG		
28501			GATCATATCG CTAGTATAGC		
28551			GCTTGCCCGT CGAACGGGCA		
28601			AGCGGGACAG TCGCCCTGTC		
28651			CCTGGATTAC GGACCTAATG		
28701			ATACAGAAAT TATGTCTTTA		
28751			ACCGTCTTCA TGGCAGAAGT		
28801			TAACATCTCT ATTGTAGAGA		
28851			GTCTACGAGA CAGATGCTCT		
28901			ACCCTCCTTA TGGGAGGAAT		
28951			ACACCTACCG TGTGGATGGC		
29001			ACTCTGTTTA TGAGACAAAT		
29051			GGCCAAAGGC CCGGTTTCCG		
29101	•		CGGGCTATTC GCCCGATAAG		
29151			TGTCTTGTGA ACAGAACACT		
29201	ACGCTTCTCT TGCGAAGAGA		CGCCGCCTGC GCGGCGGACG		
29251	TTGTCAGCTT AACAGTCGAA		GGGGTCGCCA CCCCAGCGGT		
29301	AATCCTAGGT TTAGGATCCA		TTGCGTCAGC AACGCAGTCG		
29351	TGGATTTTAA ACCTAAAATT	GGAGCCAGCC CCTCGGTCGG	TGTAATGTTA ACATTACAAT	CATTCGCAGC GTAAGCGTCG	TGAAGCTAAT ACTTCGATTA

Figure 26 AE

29401	GAGTGCACCA CTCACGTGGT	CAGAATATTT	ATGCACCACA TACGTGGTGT	GAACATGAAA CTTGTACTTT	AGCTGU T TCGACGAATA
29451	AGCGGTGTTT	TTGTTTTAAC	GCAAGTATGC CGTTCATACG	ACAAATACGA	TAAACCGTCG
29501	GTCCACTGTG	ATGTCTCATA	AATGTTACAG TTACAATGTC	AAAAGGTCCC	ATTTTCAGTA
29551	AAAACTTTTA TTTTGAAAAT	TGTATACTTT ACATATGAAA	TCCATTTTAT AGGTAAAATA	GAAATGTGCG CTTTACACGC	ACATTACCAT TGTAATGGTA
29601	CATGTACTCG	TTTGTCATAT	AGTTGTGGCC TCAACACCGG	GGGTGTTTTA	ACACACCTTT
29651	TGTGACCGTG	AAAGACGACG	ACTGCTATGC TGACGATACG	ATTAATGTCA	CGAGCGAAAC
29701	CAGACATGGG	ATGAGATATA	TAAATACAAA ATTTATGTTT	TCGTCTGCGT	CGAAATAACT
29751	CCTTTTCTTT	TACGGAATTA	TTACTAAGTT AATGATTCAA	TGTTTCGATT	ACAGTGGTGA
29801 :	TTGACGAAAT	GAGCGACGAA	GCAAAACAAA CGTTTTGTTT	AAGTTTTTCA	ATCGTAATAT
29851	TAATCTTATC	CTAAATTTGG	CCCCGGTCAT GGGGCCAGTA	AAGGACGAGT	TATGGTAAGG
29901	GGACTTGTTA	ACTGAGATAC	TGGGATATGC ACCCTATACG	AGGTCGCGAT	GTTGGAACTT
29951	CAGTCCGAAG	GACCTACAGT	GCATCTGACT CGTAGACTGA	AACCGGTCGT	GGACAGGGCG
30001	CCTAAACAAG	GTCAGGTTGA	ACAGCGACCC TGTCGCTGGG	TGGGATTGTC	TCTACTGGTT
30051	GTGTTGGTTG	CCCCCCCCC	CTACCGGACT GATGGCCTGA	ATGTAGATGG	TGTTTATGTG
30101	GGGTTCAAAG	ACGGAAACAG	AATAACTGGG TTATTGACCC	TATTGAACCC	GTACACCACC
		GCGAATACAA	ACATACGGAA	TAATAATACA	CCGAGTAGAC
		GCGTTTGCGC	GGGCTGGTGG	GTAGATATCA	GGGTAGTAAC
30251	TGCTACACCC ACGATGTGGG	AAACAATGAT TTTGTTACTA	GGAATCCATA CCTTAGGTAT	GATTGGACGG CTAACCTGCC	ACTGAAACAC TGACTTTGTG
30301	ATGTTCTTTT TACAAGAAAA	CTCTTACAGT GAGAATGTCA	ATGATTAAAT TACTAATTTA	GAGACATGAT CTCTGTACTA	TCCTCGAGTT AGGAGCTCAA

Figure 26 AF

30351	TTTATATTAC AAATATAATG	T CCTTGT ACTGGGAACA			
30401	TGCGGTTTCT ACGCCAAAGA	CACATCGAAG GTGTAGCTTC			
30451		ATTTGTCACC TAAACAGTGG			
30501		TTATCCAGTG AATAGGTCAC			GCTTTGCATA CGAAACGTAT
30551		CATCCCCAGT GTAGGGGTCA			
30601		ATTATGAAAT TAATACTTTA			
30651		GTTTTGTTCC CAAAACAAGG			
30701		CTCGTATATG GAGCATATAC			
30751		GAAGCCTGGT CTTCGGACCA			
30801		CTTAGCCCTA GAATCGGGAT			
30851		ATGCCATGAA TACGGTACTT			
30901		CAAGTTGTTG GTTCAACAAC			
30951		TCCCACCCC AGGGTGGGGG			
31001		GACACCCTAG CTGTGGGATC			
31051		AGAAAGACGC TCTTTCTGCG			
31101		TTCTGTACCA	ATTGAACGTG	GTCACGTTTT	CCCCATAGAA
31151	AACAGAGCAT	AAGCAGGCCA TTCGTCCGGT	TTCAGTGGAT	GCTGTCATTA	TEGTEGCCTG
31201	TGGCGGAATC	CTACAAGTTG GATGTTCAAC	GGTTGGTTCG	CAGTCTTTAA	CCACCAGTAC
31251	GTGGGAGAAA CACCCTCTTT	AGCCCATTAC TCGGGTAATG			

Figure 26 AG

		·			
31301 -	CTGCATTCAC	CTTGTC	AAGGACCTGA TTCCTGGACT	CCTAGAGACG	TGGGAA TAAT
	• '				
31351	AGACCCTGTG	CGGTCTCAAA	GATCTTATTC	CCTTTAACTA	ATAAAAAAAA
	TCTGGGACAC	GCCAGAGTTT	CTAGAATAAG	GGAAATTGAT	TATTTTTTT
31401	ATAATAAAGC	ATCACTTACT	TAAAATCAGT	TAGCAAATTT	CTGTCCAGTT
	TATTATTTCG	TAGTGAATGA	ATTTTAGTCA	ATCGTTTAAA	GACAGGTCAA
31451	TATTCAGCAG	CACCTCCTTG	CCCTCCTCCC	AGCTCTGGTA	TTGCAGCTTC
	ATAAGTCGTC	GTGGAGGAAC	GGGAGGAGGG	TCGAGACCAT	AACGTCGAAG
31501	CTCCTGGCTG	CAAACTTTCT	CCACAATCTA	AATGGAATGT	CAGTTTCCTC
	GAGGACCGAC	GTTTGAAAGA	GGTGTTAGAT	TTACCTTACA	GTCAAAGGAG
31551	CTGTTCCTGT	CCATCCGCAC	CCACTATCTT	CATGTTGTTG	CAGATGAAGC
71351	GACAAGGACA	GGTAGGCGTG	GGTGATAGAA	GTACAACAAC	GTCTACTTCG
21601	CCCCNNCNCC	CTCTGAAGAT	ACCTTCAACC	CCGTGTATCC	ATATGACACG
31601	CGCGTTCTGG	CAGACTTCTA	TGGAAGTTGG	GGCACATAGG	TATACTGTGC
		00000 3 COCO	GCCTTTTCTT	<b>み</b> CጥCCጥCCCጥ	TTGTATCCCC
31651	COMMCCCCAC	CICCARCIGI	CGGAAAAGAA	TGAGGAGGGA	AACATAGGGG
	C111GGCCAG	GAGG11GHCH			
31701	CAATGGGTTT	CAAGAGAGTC	CCCCTGGGGT	ACTCTCTTTG	CGCCTATCCG
	GTTACCCAAA	GTTCTCTCAG	GGGGACCCCA	TGAGAGAAAC	GCGGATAGGC
31751	እ አ ር ር ጥር ጥ እ ር ጥ	<b>ጥልሮርጥርርል</b> ልጥ	GGCATGCTTG	CGCTCAAAAT	GGGCAACGGC
31/31	TTGGAGATCA	ATGGAGGTTA	CCGTACGAAC	GCGAGTTTTA	CCCGTTGCCG
		> = = > = = = = = = = = = = = = = = = =	CAACCTTACC	TOCOBBBTG	TAACCACTGT
31801	CICICICIGG	TECTCCGGCC	GTTGGAATGG	AGGGTTTTAC	ATTGGTGACA
	_				
31851	GAGCCCACCT	CTCAAAAAAA	CCAAGTCAAA	CATAAACCTG	GAAATATCTG
	CTCGGGTGGA	GAGTTTTTTT	GGTTCAGTTT	GTATTTGGAC	CTTTATAGAC
31901	CACCCCTCAC	AGTTACCTCA	GAAGCCCTAA	CTGTGGCTGC	CGCCGCACCT
01701	GTGGGGAGTG	TCAATGGAGT	CTTCGGGATT	GACACCGACG	GCGGCGTGGA
				a	000000000000000000000000000000000000000
31951	CTAATGGTCG	CGGGCAACAC	ACTCACCATG	CAATUACAGG	CCCCGCTAAC GGGGCGATTG
32001	CGTGCACGAC	TCCAAACTTA	GCATTGCCAC	CCAAGGACCC	CTCACAGTGT
•	GCACGTGCTG	AGGTTTGAAT	CGTAACGGTG	GGTTCCTGGG	GAGTGTCACA
22051	CACAACCAAA	GCTAGCCCTG	CANACATCAG	GCCCCTCAC	CACCACCGAT
32031	CTCTTCCTTT	CGATCGGGAC	GTTTGTAGTC	CGGGGGAGTG	GTGGTGGCTA
32101	AGCAGTACCC	TTACTATCAC	TGCCTCACCC	CCTCTAACTA	CTGCCACTGG
					GACGGTGACC
32151	TAGCTTGGGC	: ATTGACTTGA	AAGAGCCCAT	TTATACACAA	AATGGAAAAC
	ATCGAACCCG	TAACTGAACI	TTCTCGGGTA	AATATGTGTT	TTACCTTTTG
30003	ma CC a CMa a a	GTACGGGGC	CCTTTGCATG	TAACAGACGA	CCTAAACACT
32201	TAGGACTAA	CATGCCCCG	GGAAACGTAC	ATTGTCTGCT	GGATTTGTGA

Figure 26 AH

32251	TTGACCGTAG AACTGGCATC	CTGGTCC	AGGTGTGACT TCCACACTGA	<sup>z</sup> ateaataat Tattattaat	CTTCCP CA GAAGGA GT
32301		ACTGGAGCCT TGACCTCGGA			
32351	TTAATGTAGC AATTACATCG	AGGAGGACTA TCCTCCTGAT			
32401		GTTATCCGTT CAATAGGCAA			
32451		CCTCTTTTTA GGAGAAAAAT			
32501		CCTTTACTTG GGAAATGAAC			
32551		TAAGCACTGC ATTCGTGACG			
32601		GCAGGAGATG CGTCCTCTAC			
32651		CCTCAAAACA GGAGTTTTGT			
32701		TGGTTCCTAA ACCAAGGATT			
32751		ACAGTAGGAA TGTCATCCTT			
32801		TCCATCTCCT AGGTAGAGGA			
32851		TGGTCTTAAC ACCAGAATTG			
32901		GCTGTTAAAG CGACAATTTC			
32951		TCTTATTATA AGAATAATAT			
33001	AATTCCTTCC TTAAGGAAGG	TGGACCCAGA ACCTGGGTCT			
33051	TGAAGGCACA ACTTCCGTGT	GCCTATACAA CGGATATGTT	ACGCTGTTGG TGCGACAACC	ATTTATGCCT TAAATACGGA	AACCTATCAG TTGGATAGTC
33101	CTTATCCAAA GAATAGGTTT	ATCTCACGGT TAGAGTGCCA			
33151	GTTTACTTAA CAAATGAATT	ACGGAGACAA TGCCTCTGTT			

Figure 26 AI

33201	AAACGGTACA TTTGCCATGT	GAAACAG GACTTTGTC	GAGACACAAC CTCTGTGTTG	TCCAAGTGCA AGGTTCACGT	TACTOT T ATGAGACA
33251	CATTTTCATG GTAAAAGTAC	GGACTGGTCT CCTGACCAGA	GGCCACAACT CCGGTGTTGA	ACATTAATGA TGTAATTACT	AATATTTGCC TTATAAACGG
33301	ACATCCTCTT TGTAGGAGAA	ACACTTTTTC TGTGAAAAAG	ATACATTGCC TATGTAACGG	CAAGAATAAA GTTCTTATTT	GAATCGTTTG CTTAGCAAAC
33351	TGTTATGTTT ACAATACAAA	CAACGTGTTT GTTGCACAAA	ATTTTTCAAT TAAAAAGTTA	TGCAGAAAAT ACGTCTTTTA	TTCAAGTCAT AAGTTCAGTA
33401	TTTTCATTCA AAAAGTAAGT	GTAGTATAGC CATCATATCG	CCCACCACCA GGGTGGTGGT	CATAGCTTAT GTATCGAATA	ACAGATCACC TGTCTAGTGG
33451	GTACCTTAAT CATGGAATTA	CAAACTCACA GTTTGAGTGT	GAACCCTAGT CTTGGGATCA	ATTCAACCTG TAAGTTGGAC	CCACCTCCCT
33501	GGGTTGTGTG	TCTCATGTGT	CAGGAAAGAG	CCCGGCTGGC GGGCCGACCG	GAATTTTTCG
33551	TAGTATAGTA	CCCATTGTCT	GTATAAGAAT	GGTGTTATAT CCACAATATA	AGGTGTGCCA
33601	AAGGACAGCT	CGGTTTGCGA	GTAGTCACTA	ATTAATAAAC TAATTATTTG	AGGGGCCCGT
33651	CGAGTGAATT	CAAGTACAGC	GACAGGTCGA	GCTGAGCCAC CGACTCGGTG	TCCGACGACA
33701	GGTTGAACGC	CAACGAATTG	CCCGCCGCTT	GGAGAAGTCC CCTCTTCAGG	TGCGGATGTA
33751	CCCCCATCTC	AGTATTAGCA	CGTAGTCCTA	AGGGCGGTGG TCCCGCCACC	ACGACGTCGT
33801	CGCGCGCTTA	TTTGACGACG	GCGGCGGCGA	CCGTCCTGCA	CCTTATGTTG
33851	TACCGTCACC	AGAGGAGTCG	CTACTAAGCG	ACCGCCCGCA TGGCGGGCGT	CGTATTCCGC
33901	GGAACAGGAG	GCCCGTGTCG	TCGCGTGGGA	•	TTTAGTCGTG
		CGTGTCGTGG	TGTTATAACA	AGTTTTAGGG	TGTCACGTTC
		GTTTCGAGTA	CCGCCCCTGG	TGTCTTGGGT	GCACCGGTAG
	TATGGTGTTC	GCGTCCATCT	AATTCACCGC	TGGGGAGTAT	AACACGCTGG TTGTGCGACC
34101	ACATAAACAT TGTATTTGTA	TACCTCTTTT ATGGAGAAAA	GGCATGTTGT	AATTCACCAC TTAAGTGGTG	CTCCCGGTAC GAGGGCCATG

Figure 26 AJ

34151	CATATAAACC GTATATTTGG	T CATTAAA A CTAATTT	CATGGCGCCA GTACCGCGGT	TCCACCACTAT AGGTGGTGGT	TCCTAA CA AGGATT ST
34201				CTGCAGGGAA GACGTCCCTT	
34251	AACAATGACA TTGTTACTGT			AACCATGGAT TTGGTACCTA	
34301				CACACGTGCA GTGTGCACGT	TACACTTCCT ATGTGAAGGA
34351	CAGGATTACA GTCCTAATGT			CATATCCCAG GTATAGGGTC	
34401				AGGGAAGACC TCCCTTCTGG	
34451				TCGGGCAGCA AGCCCGTCGT	
34501				AAAAGGAGGT TTTTCCTCCA	
34551				ATCGTGTTGG TAGCACAACC	
34601				TTTCCTGAAG AAAGGACTTC	
34651				GGTCTCGCCG CCAGAGCGGC	
34701	AGACACATCA	TCAACATCAT	ATAGGTGAGA	CTCAAAGCAT GAGTTTCGTA	GGTCCGCGGG
34751	GGACCGAAGC	CCAAGATACA	TTTGAGGAAG	ATGCGCCGCT TACGCGGCGA	CGGGACTATT
34801	GTAGGTGGTG	GCGTCTTATT	CGGTGTGGGT	GCCAACCTAC CGGTTGGATG	TGTAAGCAAG
34851	ACGCTCAGTG	TGTGCCCTCC	TCGCCCTTCT	GCTGGAAGAA CGACCTTCTT	GGTACAAAAA
		GTTTTCTAAT	AGGTTTTGGA	GTTTTACTTC	TAGATAATTC
		GGGGAGGCCA	CCGCACCAGT	TTGAGATGTC	GGTTTCTTGT
		AAACATTCTA	CAACGTGTTA	CCGAAGGTTT	TCCGTTTGCC
35051	CCCTCACGTC GGGAGTGCAG	CAAGTGGACG GTTCACCTGC	TAAAGGCTAA ATTTCCGATT	ACCCTTCAGG TGGGAAGTCC	GTGAATCTCC CACTTAGAGG

Figure 26 AK

35101	TCTATAAACA	TAGCACC	TTCAACCATG	CCCAAATAAT GGGTTTATTA	TCTCAT G
35151	CCACCTTCTC				
	GGTGGAAGAG	TTATATAGAG	ATTCGTTTAG	GGCTTATAAT	TCAGGCCGGT
35201	TTGTAAAAAT	CTGCTCCAGA	GCGCCCTCCA	CCTTCAGCCT	CAAGCAGCGA
	AACATTTTTA	GACGAGGTCT	CGCGGGAGGT	GGAAGTCGGA	GTTCGTCGCT
35251	ATCATGATTG	CAAAAATTCA	GGTTCCTCAC	AGACCTGTAT	AAGATTCAAA
	TAGTACTAAC	GTTTTTAAGT	CCAAGGAGTG	TCTGGACATA	TTCTAAGTTT
35301	AGCGGAACAT	TAACAAAAAT	ACCGCGATCC	CGTAGGTCCC	TTCGCAGGGC
	TCGCCTTGTA	ATTGTTTTTA	TGGCGCTAGG	GCATCCAGGG	AAGCGTCCCG
35351	CAGCTGAACA	TAATCGTGCA	GGTCTGCACG	GACCAGCGCG	GCCACTTCCC
	GTCGACTTGT	ATTAGCACGT	CCAGACGTGC	CTGGTCGCGC	CGGTGAAGGG
35401	CGCCAGGAAC	CATGACAAAA	GAACCCACAC	TGATTATGAC	ACGCATACTC
	GCGGTCCTTG	GTACTGTTTT	CTTGGGTGTG	ACTAATACTG	TGCGTATGAG
35451	GGAGCTATGC	TAACCAGCGT	AGCCCCGATG	TAAGCTTGTT	GCATGGGCGG
	CCTCGATACG	ATTGGTCGCA	TCGGGGCTAC	ATTCGAACAA	CGTACCCGCC
35501				ATCAGGCAAA TAGTCCGTTT	
35551	AAAAAGAAAG	CACATCGTAG	TCATGCTCAT	GCAGATAAAG	GCAGGTAAGC
	TTTTTCTTTC	GTGTAGCATC	AGTACGAGTA	CGTCTATTTC	CGTCCATTCG
35601	TCCGGAACCA	CCACAGAAAA	AGACACCATT	TTTCTCTCAA	ACATGTCTGC
	AGGCCTTGGT	GGTGTCTTTT	TCTGTGGTAA	AAAGAGAGTT	TGTACAGACG
35651	GGGTTTCTGC	ATAAACACAA	AATAAAATAA	CAAAAAAACA	TTTAAACATT
	CCCAAAGACG	TATTTGTGTT	TTATTTTATT	GTTTTTTGT	AAATTTGTAA
35701				CCTTATAAGC GGAATATTCG	
35751	CTACGGCCAT	GCCGCCGTGA	CCGTAAAAA	ACTGGTCACC	GTGATTAAAA
	GATGCCGGTA	CGGCCGCACT	GGCATTTTT	TGACCAGTGG	CACTAATTTT
35801	AGCACCACCG	ACAGCTCCTC	GGTCATGTCC	GGAGTCATAA	TGTAAGACTC
	TCGTGGTGGC	TGTCGAGGAG	CCAGTACAGG	CCTCAGTATT	ACATTCTGAG
35851	GGTAAACACA	TCAGGTTGAT	TCACATCGGT	CAGTGCTAAA	AAGCGACCGA
	CCATTTGTGT	AGTCCAACTA	AGTGTAGCCA	GTCACGATTT	TTCGCTGGCT
35901	AATAGCCCGG	GGGAATACAT	ACCCGCAGGC	GTAGAGACAA	CATTACAGCC
	TTATCGGGCC	CCCTTATGTA	TGGGCGTCCG	CATCTCTGTT	GTAATGTCGG
35951	CCCATAGGAG	GTATAACAAA	ATTAATAGGA	GAGAAAAACA	CATAAACACC
	GGGTATCCTC	CATATTGTTT	TAATTATCCT	CTCTTTTTGT	GTATTTGTGG
36001	TGAAAAACCC	TCCTGCCTAG	GCAAAATAGC	ACCCTCCCGC	TCCAGAACAA
	ACTTTTTGGG	AGGACGGATC	CGTTTTATCG	TGGGAGGGCG	AGGTCTTGTT

Figure 26 AL

36051	CATACAGCGC GTATGTCGCG	TACAGCG AAGGTGTCGC	GCAGCCATAA CGTCGGTATT	CAGTCAGCCT GTCAGTCGGA	TACCAG LA ATGGTCATTT
36101				ACACGGCACC TGTGCCGTGG	
36151				GCGAGTATAT CGCTCATATA	
36201				AACACCCAGA TTGTGGGTCT	
36251				AACCCACAAC TTGGGTGTTG	
36301	CGTCACTTCC GCAGTGAAGG	GTTTTCCCAC CAAAAGGGTG	GTTACGTCAC CAATGCAGTG	TTCCCATTIT AAGGGTAAAA	AAGAAAACTA TTCTTTTGAT
36351				CTAAAACCTA GATTTTGGAT	
36401	CCCGTTCCCA GGGCAAGGGT	GCGGGGGGGG	CACGTCACAA GTGCAGTGTT	ACTCCACCCC TGAGGTGGGG	CTCATTATCA GAGTAATAGT
					PacI
36451	TATTGGCTTC ATAACCGAAG			ATTGATGATG TAACTACTAC	
36501	ATTCGGATCT TAAGCCTAGA	GCGACGCGAG CGCTGCGCTC	GCTGGATGGC CGACCTACCG	CTTCCCCATT GAAGGGGTAA	ATGATTCTTC TACTAAGAAG
36551	TCGCTTCCGG AGCGAAGGCC	CGGCATCGGG GCCGTAGCCC	ATGCCCGCGT TACGGGCGCA	TGCAGGCCAȚ ACGTCCGGTA	GCTGTCCAGG CGACAGGTCC
36601	GTCCATCTAC	TGCTGGTAGT	CCCTGTCGAA	CAAGGCCAGC GTTCCGGTCG	TTTTCCGGTC
36651	CTTGGCATTT	TTCCGGCGCA	ACGACCGCAA	TTTCCATAGG AAAGGTATCC	GAGGCGGGGG
36701				GTCAGAGGTG CAGTCTCCAC	
36751	ACAGGACTAT TGTCCTGATA	AAAGATACCA TTTCTATGGT	GGCGTTTCCC CCGCAAAGGG	CCTGGAAGCT GGACCTTCGA	CCCTCGTGCG GGGAGCACGC
36801	CTCTCCTGTT GAGAGGACAA	CCGACCCTGC GGCTGGGACG	CGCTTACCGG GCGAATGGCC	ATACCTGTCC TATGGACAGG	GCCTTTCTCC
36851	CTTCGGGAAG GAAGCCCTTC	CGTGGCGCTT GCACCGCGAA	TCTCATAGCT AGAGTATCGA	CACGCTGTAG GTGCGACATC	GTATCTCAGT CATAGAGTCA
36901	TCGGTGTAGG AGCCACATCC	TCGTTCGCTC AGCAAGCGAG	CAAGCTGGGC GTTCGACCCG	TGTGTGCACG ACACACGTGC	AACCCCCGT TTGGGGGGCA

Figure 26 AM

36951	TCAGCCCGAC AGTCGGGCTG	TGCGCCT GCGACGCGGA	TATCCGGTAA ATAGGCCATT	CTATCGTCTT GATAGCAGAA	GAGTCO TC CTCAGG. GG
37001	CGGTAAGACA GCCATTCTGT	CGACTTATCG GCTGAATAGC	CCACTGGCAG GGTGACCGTC	CAGCCACTGG GTCGGTGACC	TAACAGGATT ATTGTCCTAA
37051	TCGTCTCGCT	CCATACATCC	CGGTGCTACA GCCACGATGT	CTCAAGAACT	TCACCACCGG
37101	ATTGATGCCG	ATGTGATCTT	GGACAGTATT CCTGTCATAA	ACCATAGACG	CGAGACGACT
37151	TCGGTCAATG	GAAGCCTTTT	AGAGTTGGTA TCTCAACCAT	CGAGAACTAG	GCCGTTTGTT
37201	TGCTGGCGAC	CATCGCCACC	TTTTTTTGTT AAAAAAAACAA	ACGTTCGTCG	TCTAATGCGC
37251	GTCTTTTTT	CCTAGAGTTC	AAGATCCTTT TTCTAGGAAA	CTAGAAAAGA	TGCCCCAGAC
37301	TGCGAGTCAC	CTTGCTTTTG	TCACGTTAAG AGTGCAATTC	CCTAAAACCA	GTACTCTAAT
37351		AGAAGTGGAT	CTAGGAAAAT	TTAGTTAGAT	TTCATATATA
37401	CTCATTTGAA	CCAGACTGTC	TTACCAATGC AATGGTTACG	AATTAGTCAC	TCCGTGGATA
37451	GAGTCGCTAG	ACAGATAAAG	GTTCATCCAT CAAGTAGGTA	TCAACGGACT	GAGGGGCAGC
37501	ACATCTATTG	ATGCTATGCC	GAGGGCTTAC CTCCCGAATG	GTAGACCGGG	GTCACGACGT
37551	TACTATGGCG	CTCTGGGTGC	CTCACCGGCT GAGTGGCCGA	GGTCTAAATA	GTCGTTATTT
37601	GGTCGGTCGG	CCTTCCCGGC	AGCGCAGAAG TCGCGTCTTC	ACCAGGACGT	TGAAATAGGC
37651	GGAGGTAGGT	CAGATAATTA	TGTTGCCGGG ACAACGGCCC	TTCGATCTCA	TTCATCAAGC
	GGTCAATTAT	CAAACGCGTT	GCAACAACGG	TAACGATGTC	GCATCGTGGT CGTAGCACCA
		AGCAAACCAT	ACCGAAGTAA	GTCGAGGCCA	AGGGTTGCTA
	GTTCCGCTCA	ATGTACTAGG	GGGTACAACA	CGTTTTTTCG	GGTTAGCTCC CCAATCGAGG
37851	TTCGGTCCTC AAGCCAGGAG	CGATCGTTGT GCTAGCAACA	CAGAAGTAAG GTCTTCATTC	TTGGCCGCAG	TGTTATCACT ACAATAGTGA

Figure 26 AN

37901	CATGGTTATG	GCACTGC	ATAATTCTCT	TACTGTCATG ATGACAGTAC	CCATCOTAA
		· ·	•		
37951	GATGCTTTTC				
	CTACGAAAAG	ACACTGACCA	CTCATGAGTT	GGTTCAGTAA	GACTCTTATC
38001				GCGTCAACAC	
	ACATACGCCG	CTGGCTCAAC	GAGAACGGGC	CGCAGTTGTG	CCCTATTATG
38051				CATCATTGGA	
				GTAGTAACCT	
38101				TGTTGAGATC	
				ACAACTCTAG	
38151				GCATCTTTTA	
				CGTAGAAAAT	
38201				AAATGCCGCA	
				TTTACGGCGT	
38251				TACTCTTCCT	
				ATGAGAAGGA	
38301				ATGAGCGGAT	
				TACTCGCCTA	
38351				TCCGCGCACA	
				AGGCGCGTGT	
38401				TTATCATGAC	
				AATAGTACTG	
38451	AAAAATAGGC				
	TTTTTATCCG	CATAGTGCTC	CGGGAAAGCA	GAAGTTCTTA	ACCTAGGCTT
		PacI			
38501	TTCTTAATTT	CTTAATTAA	(SEQ ID NO	:32)	
	AAGAATTAAA				

Figure 26 AO

## MRKAd5nef MER1063 (MRKAd5 Pre-Adenoviral Vector Containing the G2A,LLA nef Coding Region)

1	CATCATCAAT	AATATACCTT	ATTTTGGATT	GAAGCCAATA	TGATAATGAG
	GTAGTAGTTA	TTATATGGAA	TAAAACCTAA	CTTCGGTTAT	ACTATTACTC
51	GGGGTGGAGT	TTGTGACGTG	ececeece	TGGGAACGGG	GCGGGTGACG
	CCCCACCTCA	AACACTGCAC	CGCGCCCCGC	ACCCTTGCCC	CGCCCACTGC
101	TAGTAGTGTG	GCGGAAGTGT	GATGTTGCAA	GTGTGGCGGA	ACACATGTAA
	ATCATCACAC	CGCCTTCACA	CTACAACGTT	CACACCGCCT	TGTGTACATT
151	GCGACGGATG	TGGCAAAAGT	GACGTTTTTG	GTGTGCGCCG	GTGTACACAG
	CGCTGCCTAC	ACCGTTTTCA	CTGCAAAAAC	CACACGCGGC	CACATGTGTC
201	GAAGTGACAA	TTTTCGCGCG	GTTTTAGGCG	GATGTTGTAG	TAAATTTGGG
	CTTCACTGTT	AAAAGCGCGC	CAAAATCCGC	CTACAACATC	ATTTAAACCC
251	CGTAACCGAG	TAAGATTTGG	CCATTTTCGC	GGGAAAACTG	AATAAGAGGA
	GCATTGGCTC	ATTCTAAACC	GGTAAAAGCG	CCCTTTTGAC	TTATTCTCCT
301	AGTGAAATCT	GAATAATTTT	GTGTTACTCA	TAGCGCGTAA	TATTTGTCTA
	TCACTTTAGA	CTTATTAAAA	CACAATGAGT	ATCGCGCATT	ATAAACAGAT
351	GGGCCGCGGG	GACTTTGACC	GTTTACGTGG	AGACTCGCCC	AGGTGTTTTT
-	CCCGGCGCCC	CTGAAACTGG	CAAATGCACC	TCTGAGCGGG	TCCACAAAAA
401	СТСАССТСТТ	TTCCGCGTTC	CGGGTCAAAG	TTGGCGTTTT	ATTATTATAG
401	GAGTCCACAA	AAGGCGCAAG	GCCCAGTTTC	AACCGCAAAA	TAATAATATC
451	CCCCCCCGA	TCCATTGCAT	ACGTTGTATC	CATATCATAA	TATGTACATT
	CGCCGGCGCT	AGGTAACGTA	TGCAACATAG	GTATAGTATT	ATACATGTAA
501	TATATTGGCT	CATGTCCAAC	ATTACCGCCA	TGTTGACATT	GATTATTGAC
•••	ATATAACCGA	GTACAGGTTG	TAATGGCGGT	ACAACTGTAA	CTAATAACTG
551	TAGTTATTAA	TAGTAATCAA	TTACGGGGTC	ATTAGTTCAT	AGCCCATATA
	ATCAATAATT	ATCATTAGTT	AATGCCCCAG	TAATCAAGTA	TCGGGTATAT
601	TGGAGTTCCG	CGTTACATAA	CTTACGGTAA	ATGGCCCGCC	TGGCTGACCG
	ACCTCAAGGC	GCAATGTATT	GAATGCCATT	TACCGGGCGG	ACCGACTGGC
651	CCCAACGACC	CCCGCCCATT	GACGTCAATA	ATGACGTATG	TTCCCATAGT
	GGGTTGCTGG	GGGCGGGTAA	CTGCAGTTAT	TACTGCATAC	AAGGGTATCA
701	AACGCCAATA	GGGACTTTCC	ATTGACGTCA	ATGGGTGGAG	TATTTACGGT
_	TTGCGGTTAT	CCCTGAAAGG	TAACTGCAGT	TACCCACCTC	ATAAATGCCA
751	AAACTGCCCA	CTTGGCAGTA	CATCAAGTGT	ATCATATGCC	AAGTACGCCC
	TTTGACGGGT	GAACCGTCAT	GTAGTTCACA	TAGTATACGG	TTCATGCGGG
801	CCTATTGACG	TCAATGACGG	TAAATGGCCC	GCCTGGCATT	ATGCCCAGTA
	GGATAACTGC	AGTTACTGCC	ATTTACCGGG	CGGACCGTAA	TACGGGTCAT

Figure 27A

851	CATGACCTTA GTACTGGAAT	TEACTTTC ACCTGAAAG	CTACTTGGCA GATGAACCGT	GTACATCTÃC CATGTAGATG	GTATTA TA CATAAT AGT
901			CGGTTTTGGC GCCAAAACCG		
951			ATTTCCAAGT TAAAGGTTCA		
1001			AAAATCAACG TTTTAGITGC		
1051			CAAATGGGCG GTTTACCCGC		
1101			TTTAGTGAAC AAATCACTTG		
1151			TCCATAGAAG AGGTATCTTC		
1201			ATTGGAACGC TAACCTTGCG		
1251			GCAAGTGGTC CGTTCACCAG		
1301			ATGAGGAGGG TACTCCTCCC		
1351			CGCAGTGGGC GCGTCACCCG		
1401			TCACCTCCTC AGTGGAGGAG		
1451			GCCCAGGAGG CGGGTCCTCC		
1501			GAGGCCCATG CTCCGGGTAC		
1551			AGAAGGGCGG TCTTCCCGCC		
1601	CCCAGAAGAG GGGTCTTCTC				CACCCAGGGC GTGGGTCCCG
1651	TACTTCCCCG ATGAAGGGGC	ACTGGCAGAA TGACCGTCTT	CTACACCCCC GATGTGGGGG	GCCCCGGCA	TCAGGTTCCC AGTCCAAGGG
1701	CCTGACCTTC GGACTGGAAG	GGCTGGTGCT CCGACCACGA	TCAAGCTGGT AGTTCGACCA	GCCCGTGGAG CGGGCACCTC	CCCGAGAAGG GGGCTCTTCC
1751	TGGAGGAGGC ACCTCCTCCG	CAACGAGGGC GTTGCTCCCG	GAGAACAACT CTCTTGTTGA	GCGCCGCCCA CGCGGCGGGT	CCCCATGTCC GGGGTACAGG

Figure 27B

1801	CAGCACGGCA GTCGTGCCGT	AGCTCCTGGG	CGAGAAGGAG GCTCTTCCTC	GTGCTGGAGT CACGACCTCA	GGAGGT CA CCTCCAAGCT
1851	CTCCAAGCTG GAGGTTCGAC	GCCTTCCACC CGGAAGGTGG	ACGTGGCCAG TGCACCGGTC	GGAGCTGCAC CCTCGACGTG	CCCGAGTACT GGGCTCATGA
1901	ACAAGGACTG TGTTCCTGAC			CTGTGCCTTC GACACGGAAG	
1951				TCCTTGACCC AGGAACTGGG	
2001	CACTCCCACT GTGAGGGTGA			GGAAATTGCA CCTTTAACGT	
2051				GGGTGGGGCA CCCACCCGT	
2101	GGGGAGGATT CCCCTCCTAA			GCTGGGGATG CGACCCCTAC	
2151	TATGGCCGAT ATACCGGCTA			TGTGGGCGTG ACACCCGCAC	
2201	CCCTTTCTTA	ATATAAGGTG TATATTCCAC	GGGGTCTTAT CCCCAGAATA	GTAGTTTTGT CATCAAAACA	ATCTGTTTTG TAGACAAAAC
2251				CGTTTGATGG GCAAACTACC	
2301	AGCTCATATT TCGAGTATAA			TGGGCCGGG	
2351				CGTCCTGCCC	
2401				CGCCGTTGGA GCGGCAACCT	
2451	TCCGCCGCCG AGGCGGCGGC	CTTCAGCCGC GAAGTCGGCG	TGCAGCCACC ACGTCGGTGG	GCCCGCGA TDCCGCGGO	TTGTGACTGA AACACTGACT
2501				TGCAGCTTCC ACGTCGAAGG	
2551	CCCGCGATGA GGGCGCTACT	CAAGTTGACG GTTCAACTGC	GCTCTTTTGG CGAGAAAACC	CACAATTGGA GTGTTAACCT	TTCTTTGACC AAGAAACTGG
2601	CGGGAACTTA GCCCTTGAAT			TTGGATCTGC AACCTAGACG	
	TTCTGCCCTG AAGACGGGAC	TTCCGAAGGA	GGGGAGGGTT	ACGCCAAATT	TTGTATTTAT
2701	AAAAACCAGA TTTTTGGTCT				TTGCTGTCTT AACGACAGAA

Figure 27C

2751	TATTTAGGGG ATAAATCCCC	TTTTGCGCGC AAAACGCGCG			
2801		CTGTGTATTT GACACATAAA			
2851		CATGGGCATA GTACCCGTAT			
2901	TGCAGAGCTT ACGTCTCGAA	CATGCTGCGG GTACGACGCC	GGTGGTGTTG CCACCACAAC	TAGATGATCC ATCTACTAGG	AGTCGTAGCA TCAGCATCGT
2951		GCGTGGTGCC CGCACCACGG			
3001		GCCCTTGGTG CGGGAACCAC			
3051		GTGGGGATAT ÇACCCCTATA			
3101	GGCTATGTTC CCGATACAAG	CCAGCCATAT GGTCGGTATA	CCCTCCGGG GGGAGGCCCC	ATTCATGTTG TAAGTACAAC	TGCAGAACCA ACGTCTTGGT
3151	CCAGCACAGT GGTCGTGTCA	GTATCCGGTG CATAGGCCAC	CACTTGGGAA GTGAACCCTT	ATTTGTCATG TAAACAGTAC	TAGCTTAGAA ATCGAATCTT
3201	GGAAATGCGT CCTTTACGCA	GGAAGAACTT CCTTCTTGAA	GGAGACGCCC CCTCTGCGGG	TTGTGACCTC AACACTGGAG	CAAGATTTTC GTTCTAAAAG
3251		TCCATAATGA AGGTATTACT			
3301		TCTGGGATCA AGACCCTAGT			
3351		CCATTTTTAC GGTAAAAATG			
3401	TATAATGGTT ATATTACCAA	CCATCCGGCC GGTAGGCCGG	CAGGGGCGTA GTCCCCGCAT	GTTACCCTCA CAATGGGAGT	CAGATTTGCA GTCTAAACGT
3451	TTTCCCACGC AAAGGGTGCG	TTTGAGTTCA AAACTCAAGT	GATGGGGGGA CTACCCCCT	TCATGTCTAC AGTACAGATG	CTGCGGGGGG GACGCCCCGC
3501	ATGAAGAAAA TACTTCTTTT	CGGTTTCCGG GCCAAAGGCC	GGTAGGGGAG CCATCCCCTC	ATCAGCTGGG TAGTCGACCC	AAGAAAGCAG TTCTTTCGTC
3551		AGCTGCGACT TCGACGCTGA			TAAATCACAC ATTTAGTGTG
3601	CTATTACCGG GATAATGGCC	CTGCAACTGG GACGTTGACC	TAGTTAAGAG ATCAATTCTC	AGCTGCAGCT TCGACGTCGA	GCCGTCATCC CGGCAGTAGG
3651	CTGAGCAGGG GACTCGTCCC	GGGCCACTTC CCCGGTGAAG	GTTAAGCATG CAATTCGTAC	TCCCTGACTC AGGGACTGAG	GCATGTTTTC CGTACAAAAG

Figure 270

3701	CCTGACCAAA GGACTGGTTT	CCAGAA AGGCGGTCTT	GGCGCTCGCC CCGCGAGCGG	GCCCAGCGAT CGGGTCGCTA	AGCAGT TT TCGTCAAGAA
3751	GCAAGGAAGC CGTTCCTTCG	AAAGTTTTTC TTTCAAAAAG	AACGGTTTGA TTGCCAAACT	GACCGTCCGC CTGGCAGGCG	CGTAGGCATG GCATCCGTAC
3801				CGGTCCCACA GCCAGGGTGT	
3851				TCCTCGTTTC AGGAGCAAAG	
3901				CTCGTCCAGA GAGCAGGTCT	
3951				TCAGCGTAGT AGTCGCATCA	
4001				GCCAGGGTGC CGGTCCCACG	
4051				TTCGCCCTGC AAGCGGGACG	
4101	GGTAGCATTT CCATCGTAAA	GACCATGGTG CTGGTACCAC	TCATAGTCCA AGTATCAGGT	GCCCCTCCGC CGGGGAGGCG	GGCGTGGCCC CCGCACCGGG
4151				CCGCACGAGG GGCGTGCTCC	
4201				AAATACCGAT TTTATGGCTA	
4251				TCTCGCATTC AGAGCGTAAG	
4301				AGGTTTCCCC TCCAAAGGGG	
4351				CCGGTGTCCA GGCCACAGGT	
4401	CGAAAAGGCT GCTTTTCCGA	GTCCGTGTCC CAGGCACAGG	CCGTATACAG GGCATATGTC	ACTTGAGAGG TGAACTCTCC	CCTGTCCTCG GGACAGGAGC
4451	AGCGGTGTTC TCGCCACAAG	CGCGGTCCTC GCGCCAGGAG	CTCGTATAGA GAGCATATCT	AACTCGGACC TTGAGCCTGG	ACTCTGAGAC TGAGACTCTG
4501	AAAGGCTCGC TTTCCGAGCG	GTCCAGGCCA CAGGTCCGGT	GCACGAAGGA CGTGCTTCCT	GGCTAAGTGG CCGATTCACC	GAGGGGTAGC CTCCCCATCG
4551 .	GGTCGTTGTC CCAGCAACAG	CACTAGGGGG GTGATCCCCC	TCCACTCGCT AGGTGAGCGA	CCAGGGTGTG GGTCCCACAC	AAGACACATG TTCTGTGTAC
4601	TCGCCCTCTT AGCGGGAGAA	CGCCATCAAG GCCGTAGTTC	GAAGGTGATT CTTCCACTAA	GGTTTGTAGG CCAAACATCC	TGTAGGCCAC ACATCCGGTG

Figure 27E

4651	GTGACCGGGT CACTGGCCCA	CAAGGACTTC	GGGGGCTATA CCCCCGATAT	AAAGGGGGTG TTTCCCCCAC	GGGGC TT CCCCGCGCAA
4701				CGAGGGCCAG GCTCCCGGTC	
4751	CTCATGAGGG	AGACTTTTCG	CCCGTACTGA	TCTGCGCTAA AGACGCGATT	CTAACAGTCA
4801	AAGGTTTTTG	CTCCTCCTAA	ACTATAAGTG	CTGGCCCGCG GACCGGGCGC	CACTACGGAA
4851	ACTCCCACCG	GCGTAGGTAG	ACCAGTCTTT	AGACAATCTT TCTGTTAGAA	AAACAACAGT
4901	TCGAACCACC	GTTTGCTGGG	CATCTCCCGC	TTGGACAGCA AACCTGTCGT	TGAACÇGCTA
4951	CCTCGCGTCC	CAAACCAAAA	ACAGCGCTAG	GGCGCGCTCC	AACCGGCGCT
5001 .		GTGCATAAGC	GCGCGTTGCG	TGGCGGTAAG	CCCTTTCTGC
5051	CACCACGCGA	GCAGCCCGTG	GTCCACGTGC	CGCCAACCGC GCGGTTGGCG	CCAACACGTC
5101	CCACTGTTCC	AGTTGCGACC	ACCGATGGAG	TCCGCGTAGG AGGCGCATCC	GCGAGCAACC
5151	AGGTCGTCTC	CGCCGGCGGG	AACGCGCTCG	AGAATGGCGG TCTTACCGCC	ATCCCCCAGA
5201	TCGACGCAGA	GCAGGCCCCC	CAGACGCAGG	ACGGTAAAGA TGCCATTTCT	GGGGCCCGTC
5251	GTCCGCGCGC	AGCTTCATCA	GATAGAACGT	TCCTTGCAAG AGGAACGTTC	AGATCGCGGA
5301		_		CGTATGGGTT GCATACCCAA	
5351	GGGTACCGT	ACCCCACCCA	CTCGCGCCTC	GCGTACATGC CGCATGTACG	GCGTTTACAG
		TCCCCGAGAG	ACTCATAAGG	TTCTATACAT	CCCATCGTAG
	TTCCACCGCG AAGGTGGCGC	CTACGACCGC	GCGTGCATTA	GCATATCAAG	CACGCTCCCT
		GCCCTGGCTC	CAACGATGCC	CGCCCGACGA	GACGAGCCTT
5551	GACTATCTGC CTGATAGACG			GGATGATATG CCTACTATAC	

Figure 27F

5601	GGAAGACGTT CCTTCTGCAA	CTTCGACCGC	TCTGTGAGAC AGACACTCTG	CTACCGCGTC GATGGCGCAG	ACGCAG TC
5651	GAGGCGTAGG	AGTCGCGCAG	CTTGTTGACC	AGCTCGGCGG	TGACCTGCAC
	CTCCGCATCC	TCAGCGCGTC	GAACAACTGG	TCGAGCCGCC	ACTGGACGTG
5701				GATGATGTCA CTACTACAGT	
5751	GTCCCTTTTT	TTTCCACAGC	TCGCGGTTGA	GGACAAACTC	TTCGCGGTCT
	CAGGGAAAAA	AAAGGTGTCG	AGCGCCAACT	CCTGTTTGAG	AAGCGCCAGA
5801	TTCCAGTACT	CTTGGATCGG	AAACCCGTCG	GCCTCCGAAC	GGTAAGAGCC
	AAGGTCATGA	GAACCTAGCC	TTTGGGCAGC	CGGAGGCTTG	CCATTCTCGG
5851	TAGCATGTAG	AACTGGTTGA	CGGCCTGGTA	GGCGCAGCAT	CCCTTTTCTA
	ATCGTACATC	TTGACCAACT	GCCGGACCAT	CCGCGTCGTA	GGGAAAAGAT
5901	CGGGTAGCGC	GTATGCCTGC	GCGGCCTTCC	GGAGCGAGGT	GTGGGTGAGC
	GCCCATCGCG	CATACGGACG	CGCCGGAAGG	CCTCGCTCCA	CACCCACTCG
5951	GCAAAGGTGT	CCCTGACCAT	GACTTTGAGG	TACTGGTATT	TGAAGTCAGT
	CGTTTCCACA	GGGACTGGTA	CTGAAACTCC	ATGACCATAA	ACTTCAGTCA
6001	GTCGTCGCAT	CCGCCCTGCT	CCCAGAGCAA	AAAGTCCGTG	CGCTTTTTGG
	CAGCAGCGTA	GGCGGGACGA	GGGTCTCGTT	TTTCAGGCAC	GCGAAAAACC
6051	AACGCGGATT	TGGCAGGGCG	AAGGTGACAT	CGTTGAAGAG	TATCTTTCCC
	TTGCGCCTAA	ACCGTCCCGC	TTCCACTGTA	GCAACTTCTC	ATAGAAAGGG
6101	GCGCGAGGCA	TAAAGTTGCG	TGTGATGCGG	AAGGGTCCCG	GCACCTCGGA
	CGCGCTCCGT	ATTTCAACGC	ACACTACGCC	TTCCCAGGGC	CGTGGAGCCT
6151	ACGGTTGTTA	ATTACCTGGG	CGGCGAGCAC	GATCTCGTCA	AAGCCGTTGA
	TGCCAACAAT	TAATGGACCC	GCCGCTCGTG	CTAGAGCAGT	TTCGGCAACT
6201	TGTTGTGGCC	CACAATGTAA	AGTTCCAAGA	AGCGCGGGAT	GCCCTTGATG
	ACAACACCGG	GTGTTACATT	TCAAGGTTCT	TCGCGCCCTA	CGGGAACTAC
6251	GAAGGCAATT	TTTTAAGTTC	CTCGTAGGTG	AGCTCTTCAG	GGGAGCTGAG
	CTTCCGTTAA	AAAATTCAAG	GAGCATCCAC	TCGAGAAGTC	CCCTCGACTC
6301	CCCGTGCTCT	GAAAGGGCCC	AGTCTGCAAG	ATGAGGGTTG	GAAGCGACGA
	GGGCACGAGA	CTTTCCCGGG	TCAGACGTTC	TACTCCCAAC	CTTCGCTGCT
6351	ATGAGCTCCA	CAGGTCACGG	GCCATTAGCA	TTTGCAGGTG	GTCGCGAAAG
	TACTCGAGGT	GTCCAGTGCC	CGGTAATCGT	AAACGTCCAC	CAGCGCTTTC
6401	GTCCTAAACT	GGCGACCTAT	GGCCATTTTT	TCTGGGGTGA	TGCAGTAGAA
	CAGGATTTGA	CCGCTGGATA	CCGGTAAAAA	AGACCCCACT	ACGTCATCTT
6451	GGTAAGCGGG	TCTTGTTCCC	AGCGGTCCCA	TCCAAGGTTC	GCGGCTAGGT
	CCATTCGCCC	AGAACAAGGG	TCGCCAGGGT	AGGTTCCAAG	CGCCGATCCA
6501	CTCGCGCGGC	AGTCACTAGA TCAGTGATCT	GGCTCATCTC CCGAGTAGAG	CGCCGAACTT GCGGCTTGAA	CATGACCAGC GTACTGGTCG

Figure 27G

6551		CTCGACGAA			
6601		GTGACAAAGA CACTGTTTCT			
6651	GGAAGAACTG	GATCTCCCGC CTAGAGGGCG	CACCAATTGG	AGGAGTGGCT	ATTGATGTGG
6701	TGAAAGTAGA	AGTCCCTGCG	ACGGGCCGAA	CACTCGTGCT	GGCTTTTGTA
6751	AAAACGTGCG	TCAGGGACGC CAGTACTGGC	AGCGGTGCAC	GGGCTGTACA	TCCTGCACGA
6801		GTCATGACCG ACGACCGCGC			
6851		TGCTGGCGCG			
	AGCGGACCGC	CCAAACCGAC	CACCAGAAGA	TGAAGCCGAC	GAACAGGAAC
6901		TGCTCGAGGG ACGAGCTCCC			
6951		AGTCCAGATG TCAGGTCTAC			
7001		GATGGGAGCT CTACCCTCGA			
7051		AGCTCCTGCA TCGAGGACGT			
7101		CAGGTGATAC GTCCACTATG			
7151		GCAAGAGGCC CGTTCTCCGG			
7201		TGGGCCGCGG ACCCGGCGCC			
7251		CGAGCCCCCG GCTCGGGGGC	·		
7301		GGGCACGTCG CCCGTGCAGC			
7351		TGCTGGCGAA ACGACCGCTT			
7401		TGCGTGAAGA ACGCACTTCT			
7451		AGAATCAATT TCTTAGTTAA			

Figure 27H

7501	ATCTCCTGCA TAGAGGACGT	CECTCCTGA GLEGAGGACT	GTTGTCTTGA CAACAGAACT	TAGGCGATUL ATCCGCTAGA	GCCGGT T
7551			GGAGATCTCC CCTCTAGAGG		
7601			ATGCGGGCCA TACGCCCGGT		
7651			GCGGCTGTAG CGCCGACATC		
7701			GCGCGAGATT CGCGCTCTAA		
7751			CGCTGAAAGA GCGACTTTCT		
7801			GTACATAACC CATGTATTGG		
7851			CAAGGCGCTC GTTCCGCGAG		
7901			GAGTTGCGCG CTCAACGCGC		
7951	TCCAGAAGAC AGGTCTTCTG	GGATGAGCTC CCTACTCGAG	GGCGACAGTG CCGCTGTCAC	TCGCGCACCT AGCGCGTGGA	CGCGCTCAAA GCGCGAGTTT
8001			CTTCTTCAAT GAAGAAGTTA		
8051			GGCGGTGGGG		
8101			GTCGACAAAG CAGCTGTTTC		
8151			TGACGGCGCG ACTGCCGCGC		
8201	GTTGGAAGAC CAACCTTCTG	GCCGCCCGTC CGGCGGGCAG	ATGTCCCGGT TACAGGGCCA	TATGGGTTGG ATACCCAACC	CGGGGGGCTG GCCCCCGAC
8251	CCATGCGGCA GGTACGCCGT	GGGATACGGC CCCTATGCCG	GCTAACGATG CGATTGCTAC	CATCTCAACA GTAGAGTTGT	ATTGTTGTGT TAACAACACA
8301	AGGTACTCCG TCCATGAGGC	CCGCCGAGGG	ACCTGAGCGA TGGACTCGCT	GTCCGCATCG CAGGCGTAGC	ACCGGATCGG TGGCCTAGCC
8351	AAAACCTCTC TTTTGGAGAG	GAGAAAGGCG CTCTTTCCGC	TCTAACCAGT AGATTGGTCA	CACAGTCGCA GTGTCAGCGT	AGGTAGGCTG TCCATCCGAC
8401	AGCACCGTGG TCGTGGCACC	CGGGCGGCAG GCCCGCCGTC	CGGGCGGCG	TCGGGGTTGT AGCCCCAACA	TTCTGGCGGA AAGACCGCCT

Figure 27I

8451		ATGTAAT TACTACATTA			
8501		CACCATGTCC GTGGTACAGG			
8551	TCGGCCATGC	CCCAGGCTTC	GTTTTGACAT	CGGCGCAGGT	CTTTGTAGTA
8601		GGGTCCGAAG AGCCTTTCTA			
****		TCGGAAAGAT			
8651		TGCATCTATC ACGTAGATAG			
8701		TTCCTCCCAT AAGGAGGGTA			
8751		AGGTCGGCGA TCCAGCCGCT			
8801		GGTAGACTGG CCATCTGACC			
8851		TGATGGTGTA ACTACCACAT			
8901		CCCGGCTGCG GGGCCGACGC			
8951		AAATACGTAG TTTATGCATC	-		
9001		AGTGCGGCGG TCACGCCGCC			
9051		CCGGGGGCGA GGCCCCCGCT			
9101		GGACATCCAG CCTGTAGGTC			
9151		GGACGCGGTT CCTGCGCCAA	•		
9201	CATGGTCGGG GTACCAGCCC	ACGCTCTGGC TGCGAGACCG			
9251	AGACCGTGCA TCTGGCACGT	AAAGGAGAGC TTTCCTCTCG			
9301	GGATAAATTC CCTATTTAAG	GCAAGGGTAT CGTTCCCATA			
9351	ATCCGGCCGT TAGGCCGGCA	CCGCCGTGAT GGCGGCACTA			

Figure 27J

9401	AGGTGTGCGA	O AGACAA	CGGGGGAGTG	CTCCTTTTGG	CTTCCT
•	TCCACACGCT	CACTCTGTT	GCCCCCTCAC	GAGGAAAACC	GAAGGAAGGT
9451	GGCGCGGCGG	CTGCTGCGCT GACGACGCGA	AGCTTTTTTG	GCCACTGGCC	GCGCGCAGCG
9501	TAAGCGGTTA				
	ATTCGCCAAT	CCGACCTTTC	GCTTTCGTAA	TTCACCGAGC	GAGGGACATC
9551	CCGGAGGGTT				
•	GGCCTCCCAA	TAAAAGGTTC	CCAACTCAGC	GCCCTGGGGG	CCAAGCTCAG
9601	TCGGACCGGC	CGGACTGCGG	CGAACGGGGG	TTTGCCTCCC	CGTCATGCAA
	AGCCTGGCCG	GCCTGACGCC	GCTTGCCCCC	AAACGGAGGG	GCAGTACGTT
9651	GACCCCGCTT	GCAAATTCCT	CCGGAAACAG	GGACGAGCCC	CTTTTTTGCT
	CTGGGGCGAA	CCTTTAAGGA	GGCCTTTGTC	CCTGCTCGGG	GAAAAAACGA
9701	TTTCCCAGAT	GCATCCGGTG	CTGCGGCAGA	TGCGCCCCCC	TCCTCAGCAG
	AAAGGGTCTA	CGTAGGCCAC	GACGCCGTCT	ACGCGGGGGG	AGGAGTCGTC
9751	CGGCAAGAGC	AAGAGCAGCG	GCAGACATGC	AGGGCACCCT	CCCCTCCTCC
	GCCGTTCTCG	TTCTCGTCGC	CGTCTGTACG	TCCCGTGGGA	GGGGAGGAGG
9801	TACCGCGTCA	GGAGGGGCGA	CATCCGCGGT	TGACGCGGCA	GCAGATGGTG
		CCTCCCCGCT			
0951	ATTACGAACC	ררכרפרפרפר	רפפפרררפפר	ACTACCTGGA	CTTGGAGGAG
3031		GGCGCGCG			
				nenecena. co	
9901	GGCGAGGGCC	TGGCGCGGCT ACCGCGCCGA	TCCTCCCGG	AGAGGACTCG	CCGTGGGTTC
9951		AAGCGTGATA			
	CCACGTCGAC	TTCGCACTAT	GCGCACTCCG	CATGCACGGC	GCCGTCTTGG
10001		CCGCGAGGGA			
	ACAAAGCGCT	GGCGCTCCCT	CTCCTCGGGC	TCCTCTACGC	CCTAGCTTTC
10051	TTCCACGCAG				
	AAGGTGCGTC	CCGCGCTCGA	CGCCGTACCG	GACTTAGCGC	TCGCCAACGA
10101	GCGCGAGGAG	GACTTTGAGC	CCGACGCGCG	AACCGGGATT	AGTCCCGCGC
	CGCGCTCCTC	CIGAAACTCG	GGCTGCGCGC	TTGGCCCTAA	TCAGGGCGCG
10151	GCGCACACGT	GGCGGCCGCC	GACCTGGTAA	CCGCATACGA	GCAGACGGTG
	CGCGTGTGCA	CCGCCGGCGG	CTGGACCATT	GGCGTATGCT	CGTCTGCCAC
10201	AACCAGGAGA	TTAACTTTCA	AAAAAGCTTT	AACAACCACG	TGCGTACGCT
	TTGGTCCTCT	AATTGAAAGT	TTTTTCGAAA	TIGITGGIGC	ACGCATGCGA
10251	TGTGGCGCGC	GAGGAGGTGG	CTATAGGACT	GATGCATCTG	TGGGACTTTG
****	ACACCGCGCG	CTCCTCCACC	GATATCCTGA	CTACGTAGAC	ACCCTGAAAC
10201	TAAGCGCGCT	CCACCAAAAC	CCALATAGCA	<u>አ</u> ርቦቦርቦጥቦ አጥ	GGCGCAGCTYG
10201	ATTCGCGCGA	CCTCGTTTTG	GGTTTATCGT	TCGGCGAGTA	CCGCGTCGAC

Figure 27K

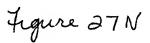
10351		T GCACAG ACGTCGTGTC			
10401		GTAGAGCCCG CATCTCGGGC			
10451		CATAGTGGTG GTATCACCAC			
10501		TCAACTATTC AGTTGATAAG		*	
10551		CATACCCCTT GTATGGGGAA			
10601		CATGCGCATG GTACGCGTAC			
10651		ATCGCAACGA TAGCGTTGCT			
10701		CTCAGCGACC GAGTCGCTGG			
10751		GGGCAGCGGC CCCGTCGCCG			
10801		TGCGCTGGGC ACGCGACCCG			
10851		GGGCTGGCGG CCCGACCGCC			
10901		ATATGACGAG TATACTGCTC			
10951		TGATGTTTCT ACTACAAAGA			
11001		CGGCGCTGCA GCCGCGACGT			
11051	CGACTGGCGC GCTGACCGCG	CAGGTCATGG GTCCAGTACC			
11101	CTGACGCGTT GACTGCGCAA	CCGGCAGCAG GGCCGTCGTC			
11151	GAAGCGGTGG CTTCGCCACC	TCCCGGCGCG AGGGCCGCGC			
11201	GATCGTAAAC CTAGCATTTG	GCGCTGGCCG CGCGACCGGC	AAAACAGGGC TTTTGTCCCG	CATCCGGCCC GTAGGCCGGG	GACGAGGCCG CTGCTCCGGC
11251	GCCTGGTCTA CGGACCAGAT	CGACGCGCTG GCTGCGCGAC			

Figure 27L

11301	AACGTGCAGA	CCTGGA	CCGGCTGGTG	GGGGATGTGC"	GCGAGG T
	TTGCACGTCT	GGTTGGACCT	GGCCGACCAC	CCCCTACACG	CGCTCCGGCA
11351	GGCGCAGCGT	GAGCGCGCGC	AGCAGCAGGG	CAACCTGGGC	TCCATGGTTG
	CCGCGTCGCA	CTCGCGCGCG	TCGTCGTCCC	GTTGGACCCG	AGGTACCAAC
11401	CACTAAACGC	CTTCCTGAGT	ACACAGCCCG	CCAACGTGCC	GCGGGGACAG
	GTGATTTGCG	GAAGGACTCA	TGTGTCGGGC	GGTTGCACGG	CGCCCTGTC
11451	GAGGACTACA	CCAACTTTGT	GAGCGCACTG	CGGCTAATGG	TGACTGAGAC
	CTCCTGATGT	GGTTGAAACA	CTCGCGTGAC	GCCGATTACC	ACTGACTCTG
11501	ACCGCAAAGT	GAGGTGTACC	AGTCTGGGCC	AGACTATTTT	TTCCAGACCA
	TGGCGTTTCA	CTCCACATGG	TCAGACCCGG	TCTGATAAAA	AAGGTCTGGT
11551	CATCTGTTCC	GGACGTCTGG	CATTTGGACT	GCCAGGCTTT CGGTCCGAAA	GTTTTTGAAC
11601	GTCCCCGACA	CCCCCCACGC	CCGAGGGTGT	CCCCTGCCCC	GCTGGCACAG
11651	TAGCTTGCTG	ACGCCCAACT	CGCGCCTGTT	GCTGCTGCTA	ATAGCGCCCT
	ATCGAACGAC	TGCGGGTTGA	GCGCGGACAA	CGACGACGAT	TATCGCGGGA
11701	TCACGGACAG	TGGCAGCGTG	TCCCGGGACA	CATACCTAGG	TCACTTGCTG
	AGTGCCTGTC	ACCGTCGCAC	AGGGCCCTGT	GTATGGATCC	AGTGAACGAC
11751	ACACTGTACC	GCGAGGCCAT	AGGTCAGGCG	CATGTGGACG	AGCATACTTT
	TGTGACATGG	CGCTCCGGTA	TCCAGTCCGC	GTACACCTGC	TCGTATGAAA
11801	CCAGGAGATT	ACAAGTGTCA	GCCGCGCGCT	GGGGCAGGAG	GACACGGGCA
	GGTCCTCTAA	TGTTCACAGT	CGGCGCGCGA	CCCCGTCCTC	CTGTGCCCGT
11851	CGGACCTCCG	AACCCTAAAC TTGGGATTTG	TACCTGCTGA ATGGACGACT	CCAACCGGCG GGTTGGCCGC	GCAGAAGATC CGTCTTCTAG
11901	CCCTCGTTGC	ACAGTTTAAA	CAGCGAGGAG	GAGCGCATTT	TGCGCTACGT
	GGGAGCAACG	TGTCAAATTT	GTCGCTCCTC	CTCGCGTAAA	ACGCGATGCA
11951	GCAGCAGAGC	GTGAGCCTTA	ACCTGATGCG	CGACGGGGTA	ACGCCCAGCG
	CGTCGTCTCG	CACTCGGAAT	TGGACTACGC	GCTGCCCCAT	TGCGGGTCGC
12001	TGGCGCTGGA	CATGACCGCG	CGCAACATGG	AACCGGGCAT	GTATGCCTCA
	ACCGCGACCT	GTACTGGCGC	GCGTTGTACC	TTGGCCCGTA	CATACGGAGT
12051	AACCGGCCGT TTGGCCGGCA	TTATCAACCG AATAGTTGGC	CCTAATGGAC GGATTACCTG	TACTTGCATC ATGAACGTAG	CGCGCCGCC
12101	CGTGAACCCC GCACTTGGGG	GAGTATTTCA CTCATAAAGT	CCAATGCCAT GGTTACGGTA	CTTGAACCCG GAACTTGGGC	CACTGGCTAC
12151	CGCCCCTGG	TTTCTACACC	GGGGGATTCG CCCCCTAAGC	AGGTGCCCGA TCCACGGGCT	GGGTAACGAT
12201	GGATTCCTCT	GGGACGACAT	AGACGACAGC	GTGTTTTCCC	CGCAACCGCA
	CCTAAGGAGA	CCCTGCTGTA	TCTGCTGTCG	CACAAAAGGG	GCGTTGGCGT

Figure 27 M

12251					GCGCTG
	CTGGGACGAT	C.AACGTTG	TCGCGCTCGT	CCGTCTCCGC	CGCGAC
12301	AGGAAAGCTT	CCGCAGGCCA	AGCAGCTTGT	CCGATCTAGG	CGCTGCGGCC
	TCCTTTCGAA	GGCGTCCGGT	TCGTCGAACA	GGCTAGATCC	GCGACGCCGG
12351	CCGCGGTCAG	ATGCTAGTAG	CCCATTTCCA	AGCTTGATAG	GGTCTCTTAC
	GGCGCCAGTC	TACGATCATC	GGGTAAAGGT	TCGAACTATC	CCAGAGAATG
12401	CAGCACTCGC	ACCACCCGCC	CGCGCCTGCT	GGGCGAGGAG	GAGTACCTAA
	GTCGTGAGCG	Tectecccc	GCGCGGACGA	CCCGCTCCTC	CTCATGGATT
12451	ACAACTCGCT	GCTGCAGCCG	CAGCGCGAAA	AAAACCTGCC	TCCGGCATTT
	TGTTGAGCGA	CGACGTCGGC	GTCGCGCTTT	TTTTGGACGG	AGGCCGTAAA
12501	CCCAACAACG	GGATAGAGAG	CCTAGTGGAC	AAGATGAGTA	GATGGAAGAC
	GGGTTGTTGC	CCTATCTCTC	GGATCACCTG	TTCTACTCAT	CTACCTTCTG
12551	GTACGCGCAG	GAGCACAGGG	ACGTGCCAGG	CCCGCGCCCG	CCCACCCGTC
	CATGCGCGTC	CTCGTGTCCC	TGCACGGTCC	GGGCGCGGGC	GGGTGGGCAG
12601	GTCAAAGGCA	CGACCGTCAG	CGGGGTCTGG	TGTGGGAGGA	CGATGACTCG
	CAGTTTCCGT	GCTGGCAGTC	GCCCCAGACC	ACACCCTCCT	GCTACTGAGC
12651	GCAGACGACA	GCAGCGTCCT	GGATTTGGGA	GGGAGTGGCA	ACCCGTTTGC
	CGTCTGCTGT	CGTCGCAGGA	CCTAAACCCT	CCCTCACCGT	TGGGCAAACG
12701	GCACCTTCGC	CCCAGGCTGG	GGAGAATGTT	TTAAAAAAAA	AAAAAGCATG
	CGTGGAAGCG	GGGTCCGACC	CCTCTTACAA	AATTTTTTTT	TTTTTCGTAC
12751		AAAAACTCAC			
	TACGTTTTAT	TITTTGAGTG	GTTCCGGTAC	CGTGGCTCGC	AACCAAAAGA
12801		TIAGTATGCG			
	ACATAAGGGG	AATCATACGC	CGCGCGCCGC	TACATACTCC	TTCCAGGAGG
12851		GAGAGTGTGG			
	AGGGAGGATG	CTCTCACACC	ACTCGCGCCG	CGGTCACCGC	CGCCGCGACC
12901		CGATGCTCCC			
		GCTACGAGGG			
12951		CCGGGGGGAG			
	GACGCCGGAT	GGCCCCCTC	TTTGTCGTAG	GCAATGAGAC	TCAACCGTGG
13001	CCTATTCGAC				
	GGATAAGCTG	TGGTGGGCAC	ACATGGACCA	CCTGTTGTTC	AGTTGCCTAC
13051	TGGCATCCCT				
		CTTGATGGTC			
13101	ATTCAAAACA				
	TAAGTTTTGT	TACTGATGTC	GGGCCCCCTC	CGTTCGTGTG	TCTGGTAGTT
	TCTTGACGAC				
	AGAACTGCTG	GCCAGCGTGA	CCCCCCCCCT	GGACTTTTGG	TAGGACGTAT



13201	CCAACATGCC GGTTGTACGC	A CACTTG	GAGTTCATGT CTCAAGTACA	TTACCAATAA AATGGTTATT	CAAATT C
13251	CGGGTGATGG GCCCACTACC	TGTCGCGCTT ACAGCGCGAA	GCCTACTAAG CGGATGATTC	GACAATCAGG CTGTTAGTCC	TGGAGCTGAA ACCTCGACTT
13301	ATACGAGTGG TATGCTCACC	GTGGAGTTCA CACCTCAAGT	CGCTGCCCGA GCGACGGGCT	GGGCAACTAC CCCGTTGATG	TCCGAGACCA AGGCTCTGGT
13351	TGACCATAGA ACTGGTATCT	CCTTATGAAC GGAATACTTG	AACGCGATCG TTGCGCTAGC	TGGAGCACTA ACCTCGTGAT	CTTGAAAGTG GAACTTTCAC
13401	GGCAGACAGA CCGTCTGTCT	ACGGGGTTCT TGCCCCAAGA	GGAAAGCGAC CCTTTCGCTG	ATCGGGGTAA TAGCCCCATT	AGTTTGACAC TCAAACTGTG
13451	GGCGTTGAAG	TCTGACCCCA	TTGACCCCGT AACTGGGGCA	GTGACCAGAA	CAGTACGGAC
13501	GGGTATATAC CCCATATATG	AAACGAAGCC TTTGCTTCGG	TTCCATCCAG AAGGTAGGTC	ACATCATTTT TGTAGTAAAA	GCTGCCAGGA CGACGGTCCT
13551	ACGCCCCACC	TGAAGTGGGT	CAGCCGCCTG GTCGGCGGAC	TCGTTGAACA	ACCCGTAGGC
13601	GTTCGCCGTT	GGGAAGGTCC	AGGGCTTTAG TCCCGAAATC	CTAGTGGATG	CTACTAGACC
13651	TCCCACCATT	GTAAGGGCGT	CTGTTGGATG GACAACCTAC	ACCTGCGGAT	GGTCCGCTCG
13701	AACTTTCTAC	TGTGGCTTGT	GGGCGGGGGT	CCGCGTCCGC	CGTCGTTGTC
13751	GTCACCGTCG	CCGCGCCTTC	AGAACTCCAA TCTTGAGGTT	GCGCCGTCGG	CGCCGTTACG
13801	TCGGCCACCT	CCTGTACTTG	GATCATGCCA CTAGTACGGT	AAGCGCCGCT	GTGGAAACGG
13851	TGTGCCCGAC	TCCTCTTCGC	GCGACTCCGG	CTTCGTCGCC	CCGAAGCTGC GGCTTCGACG
13901	GCGGGGGCGA	CGCGTTGGGC	TCCAGCTCTT	CGGAGTCTTC	AAACCGGTGA TTTGGCCACT
	AGTTTGGGGA	CTGTCTCCTG	TCGTTCTTTG	CGTCAATGTT	CCTAATAAGC GGATTATTCG
	TTACTGTCGT	GGAAGTGGGT	CATGGCGTCG	ACCATGGAAC	CATACAACTA GTATGTTGAT
	GCCGCTGGGA	GTCTGGCCTT	AGGCGAGTAC	CTGGGACGAA	TGCACTCCTG ACGTGAGGAC
14101	ACGTAACCTG TGCATTGGAC	CGGCTCGGAG GCCGAGCCTC	CAGGTCTACT GTCCAGATGA	GGTCGTTGCC CCAGCAACGG	AGACATGATG TCTGTACTAC

Tigure 270

14151					ACTTTC T TGAAAGGCCA
14201				CAAGAGCTTC GTTCTCGAAG	
14251				TTACCTCTCT AATGGAGAGA	
14301				CGCGCGCCGC	
14351				TCTCACAGAT AGAGTGTCTA	
14401				ACCGACTGAC TCGCTCACTG	
14451				AAGGCCCTGG TTCCGGGACC	
14501				AGCAAGCATG TCGTTCGTAC	
14551				TGCGCTTCCC	
14601				CACCCAGTGC GTGGGTCACG	
14651				ACGCGGCCGC TGCGCCGGCG	
14701				TGGAGGAGGC ACCTCCTCCG	
14751				GACGCGGCCA CTGCGCCGGT	
14801				GAAGAGACGG CTTCTCTGCC	
14851				CTGCCGCCCA GACGGCGGGT	
14901				GGCCGACGGG	
14951	CCCCCTCGA CCGGCGAGCT			CACTGTGCCC GTGACACGGG	
15001	GGCGACGAGC CCGCTGCTCG			CCATTAGTGC GGTAATCACG	
15051	GGTCGCAGGG CCAGCGTCCC	GCAACGTGTA CGTTGCACAT	TTGGGTGCGC AACCCACGCG	GACTCGGTTA CTGAGCCAAT	GCGGCCTGCG CGCCGGACGC

Figure 27P

15101	CGTGCCCGTG GCACGGGCAC	000000	CCCCGCGCAA GGGGCGCGTT	CTAGATTGCA GATCTAACGT	TCTTTTT-LA
15151	ACTTAGACTC TGAATCTGAG	GTACTGTTGT CATGACAACA	ATGTATCCAG TACATAGGTC	GCGCCGCCG	GCGCAACGAA CGCGTTGCTT
15201	GCTATGTCCA CGATACAGGT	ACCCCAAAAT TCGCGTTTTA	CAAAGAAGAG GTTTCTTCTC	ATGCTCCAGG TACGAGGTCC	TCATCGCGCC AGTAGCGCGG
15251	GGAGATCTAT CCTCTAGATA	GGCCCCCGA CCGGGGGGCT	AGAAGGAAGA TCTTCCTTCT	GCAGGATTAC CGTCCTAATG	AAGCCCCGAA TTCGGGGCTT
15301		CCAGTTTTTC	TTTTTCTCTC	TACTACTACT	ACTTGAACTG
15351		TIGACGACGI	GCGATGGCGC	GGGTCCGCTG	CCCATGTCAC
15401		GCGCATTTTG	CACAAAACGC	TGGGCCGTGG	TGGCATCAGA
15451		ACTCGCGAGG	TGGGCGTGGA	TGTTCGCGCA	CATACTACTC
15501	CACATGCCGC	TGCTCCTGGA	CGAACTCGTC	GCCAACGAGC CGGTTGCTCG	CCGACCCCT
15551	CAAACGGATG	CCTTTCGCCG	TATTCCTGTA	GCTGGCGTTG CGACCGCAAC	GGCGACCTGC
15601	TCCCGTTGGG	TTGTGGATCG	GATTTCGGGC	TAACACTGCA ATTGTGACGT	CGTCCACGAC
15651	GGGCGCGAAC	GTGGCAGGCT	TCTTTTCGCG	GGCCTAAAGC CCGGATTTCG	CGCTCAGACC
15701	ACTGAACCGT	GGGTGGCACG	TCGACTACCA	ACCCAAGCGC TGGGTTCGCG	GICGCTGACC
15751	TTCTACAGAA	CCTTTTTTAC	TGGCACCTTG	CTGGGCTGGA GACCCGACCT	CGGGCTCCAG
15801	GCGCACGCCG	GTTAGTTCGT	CCACCGCGGC	CCTGACCCGC	TGCAGACCGT ACGTCTGGCA
15851	CCTGCAAGTC	TATEGGTGAT	GGTCATCGTG	CAGTATTGCC GTCATAACGG	TEGEGETETE
	TCCCGTACCT	CTGTGTTTGC	AGGGGCCAAC	GGAGTCGCCA	CCCCGATGCC
		GCCAGCGACG	CCGGCGCAGG	.TTCTGGAGAT	GCCTCCACGT
16001	AACGGACCCG TTGCCTGGGC	TGGATGTTTC ACCTACAAAG	GCGTTTCAGC CGCAAAGTCG	CCCCCGCGC	CCGCGCCGTT GGCGCGGCAA

Figure 270

16051		CECCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC			
16101		CGCCTACCCC GCGGATGGGG			
16151		ACTACCCGAC TGATGGGCTG			
16201		CCAGCCCGTG GGTCGGGCAC			
16251		GCAGGACCCT CGTCCTGGGA			
16301		AAGCCGGTCT TTCGGCCAGA			
16351		TTTCCCGGTG AAAGGGCCAC			
16401		CCGGCCACGG GGCCGGTGCC			
16451		CGCGCGTCGC GCGCGCAGCG			
16501		ACTGATCGCC TGACTAGCGG			
16551		TGCAGGCGCA ACGTCCGCGT			•
16601		AATAAAAAGT TTATTTTCA			
16651		GAATGGAAGA CTTACCTTCT			
16701		CCGTTCATGG GGCAAGTACC			
16751		CGCCTTCAGC GCGGAAGTCG			
16801	TTCGGTTCCA AAGCCAAGGT	CCGTTAAGAA GGCAATTCTT	CTATGGCAGC GATACCGTCG	AAGGCCTGGA TTCCGGACCT	ACAGCAGCAC TGTCGTCGTG
16851	AGGCCAGATG TCCGGTCTAC	CTGAGGGATA GACTCCCTAT	AGTTGAAAGA TCAACTTTCT	GCAAAATTTC CGTTTTAAAG	CAACAAAAGG GTTGTTTTCC
16901	TGGTAGATGG ACCATCTACC	CCTGGCCTCT GGACCGGAGA	GGCATTAGCG CCGTAATCGC	GGGTGGTGGA CCCACCACCT	CCTGGCCAAC GGACCGGTTG
16951	CAGGCAGTGC GTCCGTCACG	AAAATAAGAT TTTTATTCTA	TAACAGTAAG ATTGTCATTC	CTTGATCCCC GAACTAGGGG	GCCCTCCCGT CGGGAGGGCA



17001	AGAGGAGCCT TCTCCTCGGA	cereccec cancecce	TGGAGACAGT ACCTCTGTCA	GTCTCCAGAG CAGAGGTCTC	GECCTV G
17051	AAAAGCGTCC	GCGCCCCGAC	AGGGAAGAAA	CTCTGGTGAC	GCAAATAGAC
	TTTTCGCAGG	CGCGGGGCTG	TCCCTTCTTT	GAGACCACTG	CGTTTATCTG
17101		CGTACGAGGA GCATGCTCCT			
17151	TCCCATCGCG AGGGTAGCGC	CCCATGGCTA GGGTACCGAT	CCGGAGTGCT GGCCTCACGA	GGGCCAGCAC	ACACCCGTAA TGTGGGCATT
17201	CGCTGGACCT GCGACCTGGA	CCCTCCCCCC	GCCGACACCC CGGCTGTGGG	AGCAGAAACC TCGTCTTTGG	TGTGCTGCCA ACACGACGGT
17251	GCCCCGACCG CCGGGCTGGC	CCGTTGTTGT	AACCCGTCCT TTGGGCAGGA	AGCCGCGCGT TCGGCGCGCA	CCCTGCGCCGC
17301		GGTCCGCGAT CCAGGCGCTA			
17351	AAAGCACACT	GAACAGCATC	GTGGGTCTGG	GGGTGCAATC	CCTGAAGCGC
	TTTCGTGTGA	CTTGTCGTAG	CACCCAGACC	CCCACGTTAG	GGACTTCGCG
17401	CGACGATGCT	TCTGATAGCT	AACGTGTCGT	ATGTGTGTCA	TCTATCCGTC
	GCTGCTACGA	AGACTATCGA	TTGCACAGCA	TACACACAGT	ACATACGCAG
17451	CATGTCGCCG GTACAGCGGC	CCAGAGGAGC GGTCTCCTCG	TGCTGAGCCG ACGACTCGGC	GCGCGCGCCC	GCTTTCCAAG CGAAAGGTTC
17501		CTTCGATGAT GAAGCTACTA			
17551	CCAGGACGCC	TCGGAGTACC	TGAGCCCCGG	GCTGGTGCAG	TTTGCCCGCG
	GGTCCTGCGG	AGCCTCATGG	ACTCGGGGCC	CGACCACGTC	AAACGGGCGC
17601	CCACCGAGAC	GTACTTCAGC	CTGAATAACA	AGTTTAGAAA	CCCCACGGTG
	GGTGGCTCTG	CATGAAGTCG	GACTTATTGT	TCAAATCTTT	GGGGTGCCAC
17651	GCGCCTACGC	ACGACGTGAC	CACAGACCGG	TCCCAGCGTT	TGACGCTGCG
	CGCGGATGCG	TGCTGCACTG	GTGTCTGGCC	AGGGTCGCAA	ACTGCGACGC
17701	GTTCATCCCT	GTGGACCGTG	AGGATACTGC	GTACTCGTAC	AAGGCGCGGT
	CAAGTAGGGA	CACCTGGCAC	TCCTATGACG	CATGAGCATG	TTCCGCGCCA
17751	TCACCCTAGC	TGTGGGTGAT	AACCGTGTGC	TGGACATGGC	TTCCACGTAC
	AGTGGGATCG	ACACCCACTA	TTGGCACACG	ACCTGTACCG	AAGGTGCATG
17801	TTTGACATCC	GCGGCGTGCT	GGACAGGGGC	CCTACTTTTA	AGCCCTACTC
	AAACTGTAGG	CGCCGCACGA	CCTGTCCCCG	GGATGAAAAT	TCGGGATGAG
17851	TGGCACTGCC	TACAACGCCC	TGGCTCCCAA	GGGTGCCCCA	AATCCTTGCG
	ACCGTGACGG	ATGTTGCGGG	ACCGAGGGTT	CCCACGGGGT	TTAGGAACGC
17901	AATGGGATGA	AGCTGCTACT	GCTCTTGAAA	TAAACCTAGA	AGAAGAGGAC
	TTACCCTACT	TCGACGATGA	CGAGAACTTT	ATTTGGATCT	TCTTCTCCTG

Figure 275

17951	GATGACAACG CTACTGTTGC	A CGAAGT TTCTGCTTCA		
18001	CGTATTTGGG GCATAAACCC	CAGGCGCCTT GTCCGCGGAA		
18051	TTCAAATAGG AAGTTTATCC	TGTCGAAGGT ACAGCTTCCA		
18101	•	CTCAAATAGG GAGTTTATCC		
18151		GGGAGAGTCC CCCTCTCAGG		
18201		TGCAAAACCC ACGTTTTGGG		
18251		AAAATGGAAA TTTTACCTTT	 	
18301		GAGGCAGCCG CTCCGTCGGC		
18351		CAGTGAAGAT GTCACTTCTA		
18401		CCACTATTAA GGTGATAATT		
18451		CCCAACAGGC GGGTTGTCCG		
18501		GTATTACAAC CATAATGTTG		
18551		AGTIGAATGC TCAACTTACG		
18601		CAGCTTTTGC GTCGAAAACG		
18651		GAATCAGGCT CTTAGTCCGA	 	
18701		ATGGAACTGA TACCTTGACT		GCTTTCCACT CGAAAGGTGA
18751	GGGAGGTGTG CCCTCCACAC	ATTAATACAG TAATTATGTC		
18801	GTCAGGAAAA CAGTCCTTTT	TGGATGGGAA ACCTACCCTT		
18851	GAAATAAGAG CTTTATTCTC	TTGGAAATAA AACCTTTATT		

Figure 27T

18901	CCTGTGGAGA GGACACCTCT	A TCCTGT TTAAAGGACA	ACTCCAACAT TGAGGTTGTA	AGCGCTGTAT TCGCGACATA	TTGCCC A AACGGGCTGT
18951	AGCTAAAGTA TCGATTTCAT	CAGTCCTTCC GTCAGGAAGG	AACGTAAAAA TTGCATTTTT	TTTCTGATAA AAAGACTATT	CCCAAACACC GGGTTTGTGG
19001				CCCGGGCTAG GGGCCCGATC	
19051				CTATATGGAC GATATACCTG	
19101				GCTACCGCTC CGATGGCGAG	
19151	GGCAATGGTC CCGTTACCAG	GCTATGTGCC CGATACACGG	CTTCCACATC GAAGGTGTAG	CAGGTGCCTC GTCCACGGAG	AGAAGTTCTT TCTTCAAGAA
19201				CTCATACACC GAGTATGTGG	
19251	TGAAGTCCTT	CCTACAATTG	TACCAAGACG	AGAGCTCCCT TCTCGAGGGA	TCCTTTACTG
19301	GATTCCCAAC	TGCCTCGGTC	GTAATTCAAA	GATAGCATTT CTATCGTAAA	CGGAAATGCG
19351	GTGGAAGAAG	GGGTACCGGG	TGTTGTGGCG	CTCCACGCTT GAGGTGCGAA	CTCCGGTACG
19401	AATCTTTGCT	GTGSTTGCTG	GTCAGGAAAT	ACGACTATCT TGCTGATAGA	GAGGCGGCGG
19451	TTGTACGAGA	TGGGATATGG	GCGGTTGCGA	ACCAACGTGC TGGTTGCACG	GGTATAGGTA
19501	GGGGAGGGCG	TTGACCCGCC	GAAAGGCGCC	CTGGGCCTTC GACCCGGAAG	TGCGCGGAAT
19551	TCTGATTCCT	TTGGGGTAGT	GACCCGAGCC	GCTACGACCC CGATGCTGGG	AATAATGTGG
19601	ATGAGACCGA	GATATGGGAT	GGATCTACCT	ACCTTTTACC TGGAAAATGG	AGTTGGTGTG
		CACCGGTAAT	GGAAACTGAG	AAGACAGTCG	ACCGGACCGT
		CGAATGGGGG	TTGCTCAAAC	TTTAATTCGC	GAGTCAACTG
		TGTTGCAACG	GGTCACATTG	TÄCTGGTTTC	TGACCAAGGA
19801	GGTACAAATG CCATGTTTAC	CTAGCTAACT GATCGATTGA	ATAACATTGG TATTGTAACC	CTACCAGGGC GATGGTCCCG	TTCTATATCC AAGATATAGG

Figure 274

19851	CAGAGAGCTA GTCTCTCGAT			CTTCCAC
19901	ATGAGCCGTC TACTCGGCAG			ACCAACAGGT TGGTTGTCCA
19951		CACCAACACA GTGGTTGTGT	 	
20001		CGAAGGACAG GCTTCCTGTC		
20051		CCGCAGTTGA GGCGTCAACT	 	
20101		TGGCGCATCC ACCGCGTAGG		
20151		CCTGGGCCAA GGACCCGGTT		
20201		CTTTTGAGGT GAAAACTCCA		
20251		GAAGTCTTTG CTTCAGAAAC		
20301		AACCGTGTAC TTGGCACATG	 	
20351		GAAGCAAGCA CTTCGTTCGT		
20401		AACTGAAAGC TTGACTTTCG		
20451		ACCTATGACA TGGATACTGT		
20501		CGCCATAGTC GCGGTATCAG	-	
20551		CCTTTGCCTG GGAAACGGAC		
20601	TGAGCCCTTT ACTCGGGAAA	GGCTTTTCTG CCGAAAAGAC		
20651	AGTACGAGTC TCATGCTCAG	ACTCCTGCGC TGAGGACGCG	 	
20701	TGTATAACGC ACATATTGCG	TGGAAAAGTC ACCTTTTCAG		
20751	CGCCTGTGGA GCGGACACCT	CTATTCTGCT GATAAGACGA		

Figure 27 V.

20801	CCCAAACTCC GGGTTTGAGG	C GATCAC GTACCTAGTG	AACCCCACCA TTGGGGTGGT	TGAACCTTAT ACTTGGAATA	TACCGG AT ATGGCCCCAT
20851				CAGCCCACCC GTCGGGTGGG	
20901				CCACTCGCCC GGTGAGCGGG	
20951				CTTTTTGTCA GAAAAACAGT	
21001	ATGTAAAAAT TACATTTTTA	AATGTACTAG TTACATGATC	AGACACTTTC TCTGTGAAAG	AATAAAGGCA TTATTTCCGT	AATGCTTTTA TTACGAAAAT
21051	AAACATGTGA	GAGCCCACTA	ATAAATGGGG	CACCCTTGCC GTGGGAACGG	CAGACGCGGC
21101	AAATTTTTAG	TTTCCCCAAG	ACGGCGCGTA	CGCTATGCGC GCGATACGCG	GTGACCGTCC
21151	CTGTGCAACG	CTATGACCAC	AAATCACGAG	CACTTAAACT GTGAATTTGA	GTCCGTGTTG
21201	CATCCGCGGC GTAGGCGCCG	AGCTCGGTGA TCGAGCCACT	AGTTTTCACT TCAAAAGTGA	CCACAGGCTG GGTGTCCGAC	CGCACCATCA GCGTGGTAGT
21251	CCAACGCGTT GGTTGCGCAA	TAGCAGGTCG ATCGTCCAGC	GGCGCCGATA CCGCGGCTAT	TCTTGAAGTC AGAACTTCAG	GCAGTTGGGG CGTCAACCCC
21301	CCTCCGCCCT GGAGGCGGGA	GCGCGCGCGCT	GTTGCGATAC CAACGCTATG	ACAGGGTTGC TGTCCCAACG	AGCACTGGAA TCGTGACCTT
21351	CACTATCAGC GTGATAGTCG	GCCGGGTGGT	GCACGCTGGC CGTGCGACCG	CAGCACGCTC GTCGTGCGAG	TTGTCGGAGA AACAGCCTCT
21401				TCAGGGCGAA AGTCCCGCTT	
21451	TTTGGTAGCT AAACCATCGA	GCCTTCCCAA CGGAAGGGTT	AAAGGGCGCG TTTCCCGCGC	TGCCCAGGCT ACGGGTCCGA	TTGAGTTGCA AACTCAACGT
21501	CTCGCACCGT GAGCGTGGCA	AGTGGCATCA TCACCGTAGT	AAAGGTGACC TTTCCACTGG	GTGCCCGGTC CACGGGCCAG	TGGGCGTTAG ACCCGCAATC
21551	GATACAGCGC CTATGTCGCG	CTGCATAAAA GACGTATTTT	GCCTTGATCT CGGAACTAGA	GCTTAAAAGC CGAATTTTCG	CACCTGAGCC GTGGACTCGG
21601	TTTGCGCCTT AAACGCGGAA	CAGAGAAGAA GTCTCTTCTT	CATGCCGCAA GTACGGCGTT	GACTTGCCGG CTGAACGGCC	AAAACTGATT TTTTGACTAA
21651	GGCCGGACAG CCGGCCTGTC	GCCGCGTCGT CGGCGCAGCA	GCACGCAGCA CGTGCGTCGT	CCTTGCGTCG GGAACGCAGC	GTGTTGGAGA CACAACCTCT
21701	TCTGCACCAC AGACGTGGTG	ATTTCGGCCC TAAAGCCGGG	CACCGGTTCT GTGGCCAAGA	TCACGATCTT AGTGCTAGAA	GGCCTTGCTA CCGGAACGAT

7, gure 27 W

21751	GACTGCTCCT	TCGCGCG	CTGCCCGTTT	TCGCTCGTCA	CATULAT
	CTGACGAGGA	AGTCGCGCGC	GACGGGCAAA	AGCGAGCAGT	GTAGGTAAAG
21801	AATCACGTGC	TCCTTATTTA	TCATAATGCT	TCCGTGTAGA	CACTTAAGCT
•		AGGAATAAAT			
21851	CGCCTTCGAT	CTCAGCGCAG	CGGTGCAGCC	ACAACGCGCA	GCCCGTGGGC
	GCGGAAGCTA	GAGTCGCGTC	GCCACGTCGG	TGTTGCGCGT	CGGGCACCCG
21901		TGTAGGTCAC	- '		
•	AGCACTACGA	ACATCCAGTG	GAGACGTTTG	CTGACGTCCA	TGCGGACGTC
21951		ATCATCGTCA			
		TAGTAGCAGT			
22001		CTCCTCC			
		CACGAGGAGC			
22051		GGTCAGGCAG			
		CCAGTCCGTC			
22101		TTGTCCATCA			
		AACAGGTAGT			
22151		GATCGGCACA			
		CTAGCCGTGT			
22201		TGGGCTCTTC			
		ACCCGAGAAG			
22251		TCTTCATTCA AGAAGTAAGT			
20201					
22301		TAGCACCGGT ATCGTGGCCA			
.00351		TTTCTTCCTC			
22351		AAAGAAGGAG			
	IGIAGAAGAG	Annonnounc	COMEMOGICE		CALC TALCE COO
22401	GCGCTCGGGC	TTGGGAGAAG	GGCGCTTCTT	TTTCTTCTTG	GGCGCAATGG
	CGCGAGCCCG	AACCCTCTTC	CCGCGAAGAA	AAAGAAGAAC	CCGCGTTACC
22451		CGCCGAGGTC			
		GCGGCTCCAG			
22501	AGCGCGTCTT	GTGATGAGTC	TTCCTCGTCC	TCGGACTCGA	TACGCCGCCT
		•			ATGCGGCGGA
22551	CATCCGCTTT	TTTGGGGGCG	CCCGGGGAGG	CGGCGGCGAC	GGGGACGGGG
		AAACCCCCGC			
22601	ACGACACGTC	CTCCATGGTT	GGGGGACGTC	GCGCCGCACC	GCGTCCGCGC
					CGCAGGCGCG
22651	TCGGGGGTGG				
	AGCCCCCACC	AAAGCGCGAC	GAGGAGAAGG	GCTGACCGGT	AAAGGAAGAG

Figure 27 X

22701	CTATAGGCAG GATATCCGTC	A GATCA TTTTTCTAGT	TGGAGTCAGT ACCTCAGTCA	CGAGAAGAAG GCTCTTCTTC	GACAGC A CTGTCGGATT
22751				CCACCGATGC GGTGGCTACG	
22801				CTTGAGGAGG GAACTCCTCC	
22851				AGACGACGAG TCTGCTGCTC	
22901				ACAACGCAGA TGTTGCGTCT	
22951				GGCGACTACC CCGCTGATGG	
23001				CCAGTGCGCC GGTCACGCGG	
23051				TCGCCATAGC AGCGGTATCG	
23101	CTTGCCTACG GAACGGATGC	AACGCCACCT TTGCGGTGGA	ATTCTCACCG TAAGAGTGGC	CGCGTACCCC GCGCATGGGG	CCAAACGCCA GGTTTGCGGT
23151	AGAAAACGGC TCTTTTGCCG	ACATGCGAGC TGTACGCTCG	CCAACCCGCG GGTTGGGCGC	CCTCAACTTC GGAGTTGAAG	TACCCCGTAT ATGGGGCATA
23201				ACATCTTTTT TGTAGAAAAA	
23251				AGCCGAGCGG TCGGCTCGCC	
23301				TATCGCCTCG ATAGCGGAGC	
23351				ACGAGAAGCG TGCTCTTCGC	
23401				AGTCACTCTG TCAGTGAGAC	
23451	GGAACTCGAG CCTTGAGCTC	GGTGACAACG CCACTGTTGC	CGCGCCTAGC GCGCGGATCG	CGTACTAAAA GCATGATTTT	CGCAGCATCG GCGTCGTAGC
23501	AGGTCACCCA TCCAGTGGGT	CTTTGCCTAC GAAACGGATG	CCGGCACTTA GGCCGTGAAT	ACCTACCCCC TGGATGGGGG	CAAGGTCATG GTTCCAGTAC
23551	AGCACAGTCA TCGTGTCAGT	TGAGTGAGCT ACTCACTCGA	GATCGTGCGC CTAGCACGCG	CGTGCGCAGC GCACGCGTCG	CCCTGGAGAG GGGACCTCTC
23601	GGATGCAAAT CCTACGTTTA	TTGCAAGAAC AACGTTCTTG	AAACAGAGGA TTTGTCTCCT	GGGCCTACCC CCCGGATGGG	GCAGTTGGCG CGTCAACCGC

Figure 27 Y

23651	ACGAGCAGCT TGCTCGTCGZ	TCGCGCGACC	CTTCAAACGC GAAGTTTGCG	GCGAGCCTGC CGCTCGGACG	CGACTT G GCTGAACCTC
23701					TGGAGCTTGA ACCTCGAACT
23751	GTGCATGCAG CACGTACGTC	CGGTTCTTTG GCCAAGAAAC	CTGACCCGGA GACTGGGCCT	GATGCAGCGC CTACGTCGCG	AAGCTAGAGG TTCGATCTCC
23801	AAACATTGCA TTTGTAACGT	CTACACCTTT GATGTGGAAA	CGACAGGGCT GCTGTCCCGA	ACGTACGCCA TGCATGCGGT	GGCCTGCAAG CCGGACGTTC
23851	ATCTCCAACG TAGAGGTTGC	TGGAGCTCTG ACCTCGAGAC	CAACCTGGTC GTTGGACCAG	TCCTACCTTG AGGATGGAAC	GAATTTTGCA CTTAAAACGT
23901		CTTGGGCAAA GAACCCGTTT			
23951		CTACGTCCGC GATGCAGGCG			
24001		CCATGGGCGT GGTACCCGCA			
24051	CAAGGAGCTG GTTCCTCGAC	CAGAAACTGC GTCTTTGACG	TAAAGCAAAA ATTTCGTTTT	CTTGAAGGAC GAACTTCCTG	CTATGGACGG GATACCTGCC
24101		GCGCTCCGTG CGCGAGGCAC			
24151		TTAAAACCCT AATTTTGGGA			
24201		CAGAACTTTA GTCTTGAAAT			
24251		CTGCTGTGCA GACGACACGT			
24301		CTCCGCCGCT GAGGCGGCGA			
24351		GCCTACCACT CGGATGGTGA			
24401		GTGTCACTGT CACAGTGACA			
24451		ATTCGCAGCT TAAGCGTCGA			
24501		GGTCCCTCGC CCAGGGAGCG			
24551		GGGGCTGTGG CCCCGACACC			

Figure 27Z

24601		ACCCACGA TGCGGGTGCT			
24651		GAGCTTACCG CTCGAATGGC			
24701		AGCCATCAAC TCGGTAGTTG			
24751		TTTACTTGGA AAATGAACCT			
24801		CCGCAGCCCT GGCGTCGGGA			
24851		CCAAAAAGAA GGTTTTTCTT			
24901		TGGGACAGTC ACCCTGTCAG			
24951		GGAAGACTGG CCTTCTGACC			
25001		CAGACGAAAC GTCTGCTTTG			
25051		AAATCGGCAA TTTAGCCGTT			
25101		GCCGGCACTG CGGCCGTGAC			
25151		CCAGGGCCGG			
25201		CAGCGCCAAG GTCGCGGTTC			
25251		TTGCTTGCAA AACGAACGTT			
25301		TCTACCATCA AGATGGTAGT			
		CATCTCTACA GTAGAGATGT			
25401		CCACACAGAA GGTGTGTCTT			
25451	AAAGCCCAAG TTTCGGGTTC	AAATCCACAG TTTAGGTGTC	CGGCGGCAGC	AGCAGGAGGA TCGTCCTCCT	GGAGCGCTGC CCTCGCGACG
25501		CAACGAACCC GTTGCTTGGG			

Figure 27. AA

25551	TTTCCCACTC AAAGGGTGAG	TGCTAT ACATACGATA		
25601		AAAAACAGGT TTTTTGTCCA		
25651		CGAAGATCAG GCTTCTAGTC	 	
25701		AATACTGCGC TTATGACGCG		
25751	TTCTCAAATT AAGAGTTTAA	TAAGCGCGAA ATTCGCGCTT		
25801		TGTTGTCAGC ACAAÇAGTCG		
25851	TACATGTGGA ATGTACACCT	GTTACCAGCC CAATGGTCGG		
25901		ACCCGAATAA TGGGCTTATT		
25951		CGGAATACGC GCCTTATGCG		
26001		CCACCACACC GGTGGTGTGG		
26051	CGCTGCCCTG GCGACGGGAC	GTGTACCAGG CACATGGTCC		
26101		CCAGGCCGAA GGTCCGGCTT		
26151		TTCGTCACAG AAGCAGTGTC		
26201		AGAGGGCGAG TCTCCCGCTC		
26251		TCTCCGTCCG AGAGGCAGGC		
26301	CGCTCTTCAT GCGAGAAGTA	TCACGCCTCG AGTGCGGAGC		
26351	CTCTGAGCCG GAGACTCGGC			
26401	TTGTGCCATC AACACGGTAG			
26451	CCGGATCAAT GGCCTAGTTA			

Figure 27 AB

26501	CTACGACTGA GATGCTGACT	A TAAGTG TACAATTCAC	GAGAGGCAGA CTCTCCGTCT	GCAACTGCGC CGTTGACGCG	CTGAAA CC GACTTTGTGG
26551	TGGTCCACTG	TCGCCGCCAC	AAGTGCTTTG	CCCGCGACTC	CGGTGAGTTT
	ACCAGGTGAC	AGCGGCGGTG	TTCACGAAAC	GGGCGCTGAG	GCCACTCAAA
26501			GGATCATATC CCTAGTATAG		
26651	CCGGCTTACC	GCCCAGGGAG	AGCTTGCCCG	TAGCCTGATT	CGGGAGTTTA
	GGCCGAATGG	CGGGTCCCTC	TCGAACGGGC	ATCGGACTAA	GCCCTCAAAT
26701	CCCAGCGCCC	CCTGCTAGTT GGACGATCAA	GAGCGGGACA CTCGCCCTGT	GGGGACCCTG CCCCTGGGAC	TGTTCTCACT ACAAGAGTGA
26751	GTGATTTGCA	ACTGTCCTAA	CCCTGGATTA	CATCAAGATC	TTTGTTGCCA
	CACTAAACGT	TGACAGGATT	GGGACCTAAT	GTAGTTCTAG	AAACAACGGT
26801	TCTCTGTGCT	GAGTATAATA	AATACAGAAA	TTAAAATATA	CTGGGGCTCC
	AGAGACACGA	CTCATATTAT	TTATGTCTTT	AATTTTATAT	GACCCCGAGG
26851	TATCGCCATC	CTGTAAACGC	CACCGTCTTC	ACCCGCCCAA	GCAAACCAAG
	ATAGCGGTAG	GACATTTGCG	GTGGCAGAAG	TGGGCGGGTT	CGTTTGGTTC
26901			TTAACATCTC AATTGTAGAG		
26951	GTTTCAACCC	AGACGGAGTG	AGTCTACGAG	AGAACCTCTC	CGAGCTCAGC
	CAAAGTTGGG	TCTGCCTCAC	TCAGATGCTC	TCTTGGAGAG	GCTCGAGTCG
27001	TACTCCATCA	GAAAAAACAC	CACCCTCCTT	ACCTGCCGGG	AACGTACGAG
	ATGAGGTAGT	CTTTTTTGTG	GTGGGAGGAA	TGGACGGCCC	TTGCATGCTC
27051	TGCGTCACCG	GCCGCTGCAC	CACACCTACC	GCCTGACCGT	AAACCAGACT
	ACGCAGTGGC	CGGCGACGTG	GTGTGGATGG	CGGACTGGCA	TTTGGTCTGA
27101	TTTTCCGGAC	AGACCTCAAT	AACTCTGTTT	ACCAGAACAG	GAGGTGAGCT
	AAAAGGCCTG	TCTGGAGTTA	TTGAGACAAA	TGGTCTTGTC	CTCCACTCGA
27151	TAGAAAACCC	TTAGGGTATT	AGGCCAAAGG	CGCAGCTACT	GTGGGGTTTA
	ATCTTTTGGG	AATCCCATAA	TCCGGTTTCC	GCGTCGATGA	CACCCCAAAT
27201	TGAACAATTC	AAGCAACTCT	ACGGGCTATT	CTAATTCAGG	TTTCTCTAGA
	ACTTGTTAAG	TTCGTTGAGA	TGCCCGATAA	GATTAAGTCC	AAAGAGATCT
27251	ATCGGGGTTG	GGGTTATTCT	CTGTCTTGTG	ATTCTCTTTA	TTCTTATACT
	TAGCCCCAAC	CCCAATAAGA	GACAGAACAC	TAAGAGAAAT	AAGAATATGA
27301	AACGCTTCTC	TGCCTAAGGC	TCGCCGCCTG	CTGTGTGCAC	ATTTGCATTT
	TTGCGAAGAG	ACGGATTCCG	AGCGGCGGAC	GACACACGTG	TAAACGTAAA
27351	ATTGTCAGCT	TTTTAAACGC	TGGGGTCGCC	ACCCAAGATG	ATTAGGTACA
	TAACAGTCGA	AAAATTTGCG	ACCCCAGCGG	TGGGTTCTAC	TAATCCATGT
27401	TAATCCTAGG	TTTACTCACC	CTTGCGTCAG	CCCACGGTAC	CACCCAAAAG
	ATTAGGATCC	AAATGAGTGG	GAACGCAGTC	GGGTGCCATG	GTGGGTTTTC

Ligure 27AC

27451			CTGTAATGTT GACATTACAA	CTGAAG A GACTTCGATT
27501			AATGCACCAC TTACGTGGTG	
27551			GGCAAGTATG CCGTTCATAC	 
27601			TAATGTTACA ATTACAATGT	 
<b>27651</b>			TTCCATTTTA AAGGTAAAAT	 
27701			AAGTTGTGGC TTCAACACCG	 
27751			CACTGCTATG GTGACGATAC	 
27801		-	TTAAATACAA AATTTATGTT	
27851			TTTACTAAGT AAATGATTCA	 
27901			TGCAAAACAA ACGTTTTGTT	
27951			CCCCCGGTCA GGGGGCCAGT	
28001			GTGGGATATG CACCCTATAC	
28051			AGCATCTGAC TCGTAGACTG	
28101			TACAGCGACC ATGTCGCTGG	
28151			GCTACCGGAC CGATGGCCTG	
28201	CCCCAAGTTT GGGGTTCAAA		CAATAACTGG GTTATTGACC	
28251	GTTCTCCATA CAAGAGGTAT		TTGTATGCCT AACATACGGA	 
28301	GCTGCCTAAA CGACGGATTT			TCCCATCATT AGGGTAGTAA
28351	GTGCTACACC CACGATGTGG		TGGAATCCAT ACCTTAGGTA	

Figure 27AD

28401	CATGTTCTTT GTACAAGAAA	TTACAG AGAGAATGTC	TATGATTAAA ATACTAATTT	TGAGACATGÁ ACTCTGTACT	TTCCTC T AAGGAGCTCA
28451	TTTTATATTA TAATATAAAA	CTGACCCTTG GACTGGGAAC	TTGCGCTTTT AACGCGAAAA	TTGTGCGTGC AACACGCACG	TCCACATTGG AGGTGTAACC
28501	CTGCGGTTTC GACGCCAAAG	TCACATCGAA AGTGTAGCTT	GTAGACTGCA CATCTGACGT	TTCCAGCCTT AAGGTCGGAA	CACAGTCTAT GTGTCAGATA
28551	TTGCTTTACG AACGAAATGC	GATTTGTCAC CTAAACAGTG	CCTCACGCTC GGAGTGCGAG	ATCTGCAGCC TAGACGTCGG	TCATCACTGT AGTAGTGACA
28601	CCAGTAGCGG	AAATAGGTCA	GCATTGACTG CGTAACTGAC	CCAGACACAC	GCGAAACGTA
28651	TAGAGTCTGT	GGTAGGGGTC	TACAGGGACA ATGTCCCTGT	CCTGATATCG	ACTCGAAGAA
28701	TCTTAAGAAA	TTAATACTTT	TTTACTGTGA AAATGACACT	GAAAAGACGA	CTAATAAACG
28751	TGGGATAGAC	GCAAAACAAG	CCCGACCTCC GGGCTGGAGG	TTCGGAGTTT	CTGTATATAG
28801	TACGTCTAAG	TGAGCATATA	GGAATATTCC CCTTATAAGG	TTCAACGATG	TTACTTTTTT
28851	CGCTAGAAAG	GCTTCGGACC	TTATATGCAA AATATACGTT	AGTAGAGACA	ATACCACAAG
28901	ACGTCATGGT	AGAATCGGGA	AGCTATATAT TCGATATATA	GGGATGGAAC	TGTAACCGAC
28951	CTTGCGTTAT	CTACGGTACT	ACCACCCAAC TGGTGGGTTG	AAAGGGGCGC	GGGCGATACG
29001	AAGGTGACGT	TGTTCAACAA	CGGCCGCCGA	AACAGGGTCG	CAATCAGCCT GTTAGTCGGA
29051	GCGGGTGGAA	GAGGGTGGGG	GTGACTTTAG	TCGATGAAAT	ATCTAACAGG TAGATTGTCC
29101	TCCTCTACTG	ACTGTGGGAT	CTAGATCITT	ACCTGCCTTA	TATTACAGAG ATAATGTCTC
	GTCGCGGACG	ATCTTTCTGC	GTCCCGTCGC	CGGCTCGTTG	AGCGCATGAA TCGCGTACTT
	AGTTCTCGAG	GTTCTGTACC	AATTGAACGT	GGTCACGTTT	AGGGGTATCT TCCCCATAGA
29251	TTTGTCTCGT AAACAGAGCA	AAAGCAGGCC TTTCGTCCGG	AAAGTCACCT TTTCAGTGGA	ACGACAGTAA TGCTGTCATT	TACCACCGGA ATGGTGGCCT
29301	CACCGCCTTA CTGCCGGAAT	GCTACAAGTT CGATGTTCAA	GCCAACCAAG CGGTTGGTTC	CGTCAGAAAT GCAGTCTTTA	TGGTGGTCAT

Figure 27 AE

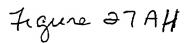
29351		A CCATTA TTCGGGTAAT			
29401		CTCACCTTGT GAGTGGAACA			
29451	AAGACCCTGT TTCTGGGACA	GCGGTCTCAA CGCCAGAGTT			
29501		CATCACTTAC GTAGTGAATG			
29551		GCACCTCCTT CGTGGAGGAA			
29601		GCAAACTTTC CGTTTGAAAG			
29651		TCCATCCGCA AGGTAGGCGT			
29701		CGTCTGAAGA GCAGACTTCT			
29751		CCTCCAACTG GGAGGTTGAC			
29801	••••	TCAAGAGAGT AGTTCTCTCA			
29851		TTACCTCCAA AATGGAGGTT			
29901		GACGAGGCCG CTGCTCCGGC			
29951		TCTCAAAAA AGAGTTTTTT			
30001		CAGTTACCTC GTCAATGGAG			
30051		GCGGGCAACA CGCCCGTTGT			
		GAGGTTTGAA	TCGTAACGGT	GGGTTCCTGG	GGAGTGTCAC
	TCAGAAGGAA AGTCTTCCTT	TCGATCGGGA	CGTTTGTAGT	CCGGGGGAGT	GCTGCTGGCT
	TAGCAGTACC ATCGTCATGG	GAATGATAGT	GACGGAGTGG	GGGAGATTGA	TGACGGTGAC
30251	GTAGCTTGGG CATCGAACCC				

Figure 27 AF

30301	CTAGGACTAA GATCCTGATT	A CGGGGC TCGCCCCG	TCCTTTGCAT AGGAAACGTA	GTAACAGAČG CATTGTCTGC	TGGATTIOTG
30351				TATTAATAAT ATTATTAATA	
30401				ATTCACAAGG TAAGTGTTCC	
30451				TCTCAAAACA AGAGTTTTGT	
30501	ACTTGATGTT TGAACTACAA			AAACCAACTA TTTGGTTGAT	
30551				CCCACAACTT GGGTGTTGAA	
30601				TCAAACAATT AGTTTGTTAA	
30651				GATGTTTGAC CTACAAACTG	
30701				TTGGTTCACC AACCAAGTGG	
30751				CATGGCCTAG GTACCGGATC	
30801				TGGCCTTAGT ACCGGAATCA	
30851				ATGATAAGCT TACTATTCGA	
30901				CTAAATGCAG GATTTACGTC	
30951				CAGTCAAATA GTCAGTTTAT	
31001				CTCCAATATC GAGGTTATAG	
31051	CAAAGTGCTC GTTTCACGAG	ATCTTATTAT TAGAATAATA	AAGATTTGAC TTCTAAACTG	GAAAATGGAG CTTTTACCTC	TGCTACTAAA ACGATGATTT
31101	CAATTCCTTC GTTAAGGAAG	CTGGACCCAG GACCTGGGTC	AATATTGGAA TTATAACCTT	CTTTAGAAAT GAAATCTTTA	GGAGATCTTA CCTCTAGAAT
31151	CTGAAGGCAC GACTTCCGTG	AGCCTATACA TCGGATATGT	AACGCTGTTG TTGCGACAAC	GATTTÀTGCC CTAAATACGG	TAACCTATCA ATTGGATAGT
31201	GCTTATCCAA CGAATAGGTT	AATCTCACGG TTAGAGTGCC	TAAAACTGCC ATTTTGACGG	AAAAGTAACA TTTTCATTGT	TTGTCAGTCA AACAGTCAGT

Figure 27 AG

31251		ANGGAGACA TIGCCTCTGT			
31301	TAAACGGTAC ATTTGCCATG	ACAGGAAACA TGTCCTTTGT			
31351	TCATTTTCAT AGTAAAAGTA	GGGACTGGTC CCCTGACCAG			
31401	•	TACACTTTTT ATGTGAAAAA			
31451		TCAACGTGTT AGTTGCACAA			
31501	TTTTTCATTC AAAAAGTAAG	AGTAGTATAG TCATCATATC			
31551	• • • • • • • • • • • • • • • • • • • •	TCAAACTCAC AGTTTGAGTG			
31601		CAGAGTACAC GTCTCATGTG			
31651		TGGGTAACAG ACCCATTGTC			
31701	TTTCCTGTCG AAAGGACAGC	AGCCAAACGC TCGGTTTGCG			
31751		AGTTCATGTC TCAAGTACAG			
31801		GGTTGCTTAA CCAACGAATT			
31851		GTCATAATCG CAGTATTAGC			
31901		TAAACTGCTG ATTTGACGAC			
31951		GTCTCCTCAG CAGAGGAGTC			
32001	GCCTTGTCCT CGGAACAGGA	CCGGGCACAG GGCCCGTGTC	CAGCGCACCC GTCGCGTGGG	TGATCTCACT ACTAGAGTGA	TAAATCAGCA ATTTAGTCGT
32051	CAGTAACTGC GTCATTGACG	AGCACAGCAC TCGTGTCGTG	CACAATATTG GTGTTATAAC	TTCAAAATCC AAGTTTTAGG	CACAGTGCAA GTGTCACGTT
32101	GGCGCTGTAT CCGCGACATA	CCAAAGCTCA GGTTTCGAGT	TGGCGGGGAC ACCGCCCCTG	CACAGAACCC GTGTCTTGGG	ACGTGGCCAT TGCACCGGTA
32151	CATACCACAA GTATGGTGTT	GCGCAGGTAG CGCGTCCATC	ATTAAGTGGC TAATTCACCG	GACCCCTCAT CTGGGGAGTA	AAACACGCTG TTTGTGCGAC



32201			TGGCATGTTG ACCGTACAAC		
32251			ACATGGCGCC TGTACCGCGG		
32301			CCGGCTATAC GGCCGATATG		
32351			CCAGGACTCG GGTCCTGAGC		
32401			CACAACACAG GTGTTGTGTC		
32451			CGCGTTAGAA GCGCAATCTT		
32501			TCCCACACTG AGGGTGTGAC		
32551			AAGTGTTACA TTCACAATGT		
32601			GTTTCTGTCT CAAAGACAGA		
32651			AGACAACCGA TCTGTTGGCT		
32701			ACGTAGTCAT TGCATCAGTA		
32751		-	TCTGCGTCTC AGACGCAGAG		
32801			ATATCCACTC TATAGGTGAG		
32851	~~~~~~~		TAAACTCCTT ATTTGAGGAA		
32901			AGCCACACCC TCGGTGTGGG		
32951	CTGCGAGTCA GACGCTCAGT	CACACGGGAG GTGTGCCCTC	GAGCGGGAAG CTCGCCCTTC	AGCTGGAAGA TCGACCTTCT	ACCATGTTTT TGGTACAAAA
33001	TTTTTTTATT AAAAAAATAA		ATCCAAAACC TAGGTTTTGG		
33051	GTGAACGCGC CACTTGCGCG	TCCCCTCCGG AGGGGAGGCC	TGGCGTGGTC ACCGCACCAG	AAACTCTACA TTTGAGATGT	GCCAAAGAAC CGGTTTCTTG
33101	AGATAATGGC TCTATTACCG		TGTTGCACAA ACAACGTGTT		

Figure 27 AI

33151	GCCCTCACGT CGGGAGTGCA	GTGGAC GTCACCTG	GTAAAGGCTA CATTTCCGAT	AACCCTTCAG TTGGGAAGTC	TGTGAAL TC CCACTITUTAG
33201		ATTCCAGCAC TAAGGTCGTG			
33251		CAATATATCT GTTATATAGA			
33301		TCTGCTCCAG AGACGAGGTC			
33351		GCAAAAATTC CGTTTTTAAG			
33401		TTAACAAAA AATTGTTTTT			
33451		ATAATCGTGC TATTAGCACG			
33501	CCGCCAGGAA GGCGGTCCTT	CCATGACAAA GGTACTGTTT	AGAACCCACA TCTTGGGTGT	CTGATTATGA GACTAATACT	CACGCATACT GTGCGTATGA
33551		CTAACCAGCG GATTGGTCGC			
33601		ATGCAAGGTG TACGTTCCAC			
33651		GCACATCGTA CGTGTAGCAT			
33701		ACCACAGAAA TGGTGTCTTT			
33751		CATAAACACA GTATTTGTGT			
3380i		TCTTACAACA AGAATGTTGT			
33851		TGCCGGCGTG ACGGCCGCAC			
33901	AAGCACCACC TTCGTGGTGG	GACAGCTCCT CTGTCGAGGA	CGGTCATGTC GCCAGTACAG	CGGAGTCATA GCCTCAGTAT	ATGTAAGACT TACATTCTGA
33951	CGGTAAACAC GCCATTTGTG	ATCAGGTTGA TAGTCCAACT	TTCACATCGG AAGTGTAGCC	TCAGTGCTAA AGTCACGATT	AAAGCGACCG TTTCGCTGGC
34001	AAATAGCCCG TTTATCGGGC	GGGGAATACA CCCCTTATGT	TACCCGCAGG ATGGGCGTCC	CGTAGAGACA GCATCTCTGT	ACATTACAGC TGTAATGTCG
34051	CCCCATAGGA GGGGTATCCT	GGTATAACAA CCATATTGTT	AATTAATAGG TTAATTATCC	AGAGAAAAAC TCTCTTTTG	ACATAAACAC TGTATTTGTG

Figure 27 AJ

34101	CTGAAAAACC GACTTTTTGG	CTATTGCCTA G. ACGGAT	GGCAAAATAG CCGTTTTATC	CACCCTCC@G GTGGGAGGGC	CACCTO T
34151	ACATACAGCG TGTATGTCGC	CTTCCACAGC GAAGGTGTCG	GGCAGCCATA CCGTCGGTAT	ACAGTCAGCC TGTCAGTCGG	TTACCAGTAA AATGGTCATT
34201	AAAAGAAAAC TTTTCTTTTG	CTATTAAAAA GATAATTTTT	AACACCACTC TTGTGGTGAG	GACACGCCAC CTGTGCCGTG	CAGCTCAATC GTCGAGTTAG
34251	AGTCACAGTG TCAGTGTCAC	TAAAAAAGGG ATTTTTTCCC	CCAAGTGCAG GGTTCACGTC	AGCGAGTATA TCGCTCATAT	TATAGGACTA ATATCCTGAT
34301	AAAAATGACG TTTTTACTGC	TAACGGTTAA ATTGCCAATT	AGTCCACAAA TCAGGTGTTT	AAACACCCAG TTTGTGGGTC	AAAACCGCAC TTTTGGCGTG
34351	GCGAACCTAC CGCTTGGATG	GCCCAGAAAC CGGGTCTTTG	GAAAGCCAAA CTTTCGGTTT	AAACCCACAA TTTGGGTGTT	CTTCCTCAAA GAAGGAGTTT
34401	TCGTCACTTC AGCAGTGAAG	CGTTTTCCCA GCAAAAGGGT	CGTTACGTCA GCAATGCAGT	CTTCCCATTT GAAGGGTAAA	TAAGAAAACT ATTCTTTTGA
34451	ACAATTCCCA TGTTAAGGGT	ACACATACAA TGTGTATGTT	GTTACTCCGC CAATGAGGCG	CCTAAAACCT GGATTTTGGA	ACGTCACCCG TGCAGTGGGC
34501	CCCCGTTCCC GGGGCAAGGG	ACGCCCCGCG TGCGGGGGCGC	CCACGTCACA GGTGCAGTGT	AACTCCACCC TTGAGGTGGG	CCTCATTATC GGAGTAATAG
					PacI
34551	ATATTGGCTT	CAATCCAAAA	TAAGGTATAT	TATTGATGAT	GTTAATTAAG
34551	ATATTGGCTT TATAACCGAA	CAATCCAAAA GTTAGGTTTT	TAAGGTATAT ATTCCATATA	TATTGATGAT ATAACTACTA	CAATTAATTC
34551 34601	TATAACCGAA AATTCGGATC	GTTAGGTTTT TGCGACGCGA	ATTCCATATA GGCTGGATGG	ATAACTACTA CCTTCCCCAT	CAATTAATTC
	TATAACCGAA AATTCGGATC TTAAGCCTAG	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT	ATTCCATATA GGCTGGATGG CCGACCTACC	ATAACTACTA CCTTCCCCAT GGAAGGGGTA	CAATTAATTC TATGATTCTT ATACTAAGAA
	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG	GTTAGGTTTT  TGCGACGCGA ACGCTGCGCT GCGGCATCGG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG
34601	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC
34601	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT	GTTAGGTTTT  TGCGACGCGA ACGCTGCGCT  GCGGCATCGG CGCCGTAGCC  GACGACCATC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA
34601 34651 34701	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA	GTTAGGTTTT  TGCGACGCGA ACGCTGCGCT  GCGGCATCGG CGCCGTAGCC  GACGACCATC CTGCTGGTAG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT
34601 34651	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA	GTTAGGTTTT  TGCGACGCGA ACGCTGCGCT  GCGGCATCGG CGCCGTAGCC  GACGACCATC CTGCTGGTAG  AAAGGCCGCG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC
34601 34651 34701	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA  GGAACCGTAA CCTTGGCATT	GTTAGGTTTT  TGCGACGCGA ACGCTGCGCT  GCGGCATCGG CGCCGTAGCC  GACGACCATC CTGCTGGTAG  AAAGGCCGCG TTTCCGGCGC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG
34601 34651 34701 34751 34801	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC  GCAGGTAGAT CGTCCATCTA  CGTACCGTAA CCTTGGCATT  CCTGACGAGC GGACTGCTCG GACAGGACTA	GTTAGGTTTT  TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC ATCACAAAAA TAGTGTTTTT TAAAGATACC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA AGCTGCGAGT AGCTGCGAGT AGCTGCGAGT	ATAACTACTA  CCTTCCCCAT GGAAGGGGTA  TTGCAGGCCA AACGTCCGGT  TCAAGGCCAG AGTTCCGGTC  TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA  CCCTGGAAGGC	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG
34601 34651 34701 34751 34801 34851	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC  GCAGGTAGAT CGTCCATCTA  CGTACCGATC  CCTGACGAGC GGACTGCTCG  GACAGGACTA CTGTCCTGAT  CTGTCCTGAT	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC ATCACAAAAA TAGTGTTTT TAAAGATACC ATTTCTATGG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA AGCTGCGAGT AGCTGCGAGT CCGCGAAAGG CCGCTTACCG	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GCGAAACCC CCGCTTTGGG TCCCTCGTGC
34601 34651 34701 34751 34801 34851 34901	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC  GCAGGTAGAT CGTCCATCTA  CGTACCATCTA  CCTGACGAGC GGACTGCTCG  GACAGGACTA CTGTCCTGAT  CTGTCCTGAT  CTGTCCTGAT  CCTCCTGAT  CCTCCTGAT  CCTCCTGAT  CCTCCTGAT  CCTCCTGAT  CCTCCTGAT  CCTCCTGAT  CCTCCTGAT  CCTCCCTGAT  CCTCCCTGAT  CCTCCCTGAT  CCTTCCGGGAA	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG ATCACAAAAA TAGTGTTTT TAAAGATACC ATTTCTATGG TCCGACCCTG AGGCTGGCAC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA AGCTGCGAGT AGCGCTCA AGCGCTTACCG CCGCTTACCG GGCGAATGGC TTCTCATAGC	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC CTATGGACAG TCACGCTGTA	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GCGAAACCC CCGCTTTGGG TCCCTCGTGC AGGGAGCACG

GA GETTCGACCC GACACACETE CT.

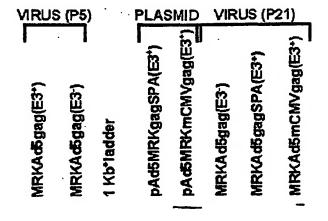
35051		CCGCTGCGCC GGCGACGCGG		
35101	CCGGTAAGAC			
55255		TGCTGAATAG		
35151		AGGTATGTAG TCCATACATC		
35201		CTACACTAGA GATGTGATCT		 
35251		CCTTCGGAAA GGAAGCCTTT		 
35301		GGTAGCGGTG CCATCGCCAC		
35351		AGGATCTCAA TCCTAGAGTT		
35401		GGAACGAAAA CCTTGCTTTT		 
35451		ATCTTCACCT TAGAAGTGGA		 
35501		TGGTCTGACA ACCAGACTGT		
35551		CTGTCTATTT GACAGATAAA	• •	 
35601		CTACGATACG GATGCTATGC		
35651		CGAGACCCAC GCTCTGGGTG		
35701		CGGAAGGGCC GCCTTCCCGG		
35751	GCCTCCATCC CGGAGGTAGG	AGTCTATTAA TCAGATAATT		
35801	GCCAGTTAAT CGGTCAATTA	AGTTTGCGCA TCAAACGCGT		
35851	TGTCACGCTC ACAGTGCGAG	GTCGTTTGGT CAGCAAACCA		
35901	TCAAGGCGAG AGTTCCGCTC	TTACATGATC AATGTACTAG		
35951	CTTCGGTCCT GAAGCCAGGA	CCGATCGTTG GGCTAGCAAC		

Figure 2 7AL

36001	TCATGGTTAT	AGCACTG	CATAATTCTC	TTACTGTCAT	GCCATC. TA
	AGTACCAATA	CCGTCGTGAC	GTATTAAGAG	AATGACAGTA	CGGTAGGCAT
36051	AGATGCTTTT	CTGTGACTGG	TGAGTACTCA	ACCAAGTCAT	TCTGAGAATA
	TCTACGAAAA	GACACTGACC	ACTCATGAGT	TGGTTCAGTA	AGACTCTTAT
•					
36101				GGCGTCAACA	
	CACATACGCC	GCTGGCTCAA	CGAGAACGGG	CCGCAGTTGT	GCCCTATTAT
36151				TCATCATTGG	
	GGCGCGGTGT	ATCGTCTTGA	AATTTTCACG	AGTAGTAACC	TTTTGCAAGA
		•			
36201	TCGGGGCGAA	AACTCTCAAG	GATCTTACCG	CTGTTGAGAT	CCAGTTCGAT
	AGCCCCGCTT	TTGAGAGTTC	CTAGAATGGC	GACAACTCTA	GGTCAAGCTA
				•	
36251				AGCATCTTTT	
	CATTGGGTGA	GCACGTGGGT	TGACTAGAAG	TCGTAGAAAA	TGAAAGTGGT
36301	GCGTTTCTGG				
	CGCAAAGACC	CACTCGTTTT	TGTCCTTCCG	TTTTACGGCG	TTTTTTCCCT
		•			mmmmc2.2003
36351	ATAAGGGCGA				
	TATTCCCGCT	GTGCCTTTAC	AACTTATGAG	TATGAGAAGG	AAAAAGTTAT
			omma mmomom	C) DC) CCCC)	መአርአውአውጥር
36401				CATGAGCGGA	
	AATAACTTCG	TAAATAGTCC	CAATAACAGA	GTACTCGCCT	ATGIAIAAAC
26453	>> mom> mmm>	C	CA A AMA CCCC	TTCCGCGCAC	ATTITICCCCCA
36451				AAGGCGCGTG	
	TTACATAAAT	CTTTTATT	GITIATCCCC	ANGGEGEGIG	17414GGGGGC1
36501	********	このこと ここのこのこの	אכאאארראיי	ATTATCATGA	СТООДЕТТА
30201	MMMC A CCCMC	CIGACGICIA	TOTALCCE A	TAATAGTACT	GTAATTGGAT
•	ITICACGGIG	GACTGCAGAT	10111001727		•
36551	ממתמממת	ССТАТСАССА	GGCCCTTTCG	TCTTCAAGAA	TTGGATCCGA
30331				AGAAGTTCTT	
		PacI			
36601	ATTCTTAATT	TCTTAATTAA	(SEQ ID NO	:34)	
			(450 TD NO		

TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Figure 27AM



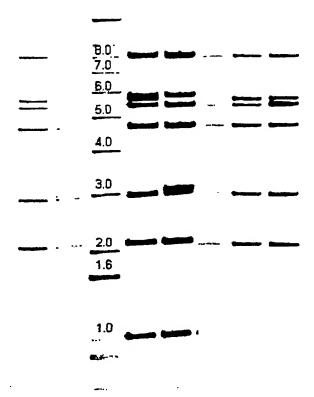


FIGURE 28

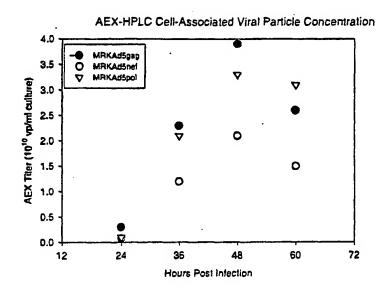


FIGURE 29A

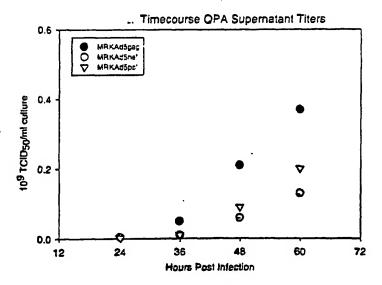


FIGURE 29B

atg Met 1	gat Asp	gca Ala	atg Met	aag Lys 5	aga Arg	ggg Gly	ctc Leu	tgc Cys	tgt Cys '10	gtg Val	ctg Leu	ctg Leu	ctg Leu	tgt Cys 15	gga Gly	48
gca Ala	gtc Val	ttc Phe	gtt Val 20	tcg Ser	ccc Pro	agc Ser	gag Glu	atc Ile 25	tcc Ser	att Ile	gtg Val	tgg Trp	gcc Ala 30	tcc Ser	agg Arg	96
gag Glu	ctg Leu	gag Glu 35	agg Arg	ttt Phe	gct Ala	gtg Val	aac Asn 40	cct Pro	ggc Gly	ctg Leu	ctg Leu	gag Glu 45	acc Thr	tct Ser	gag Glu	144
Gly aaa	tgc Cys 50	agg Arg	cag Gln	atc Ile	ctg Leu	ggc Gly 55	cag Gln	ctc Leu	cag Gln	ccc Pro	tcc Ser 60	ctg Leu	caa Gln	aca Thr	ggc Gly	192
tct Ser 65	gag Glu	gag Glu	ctg Leu	agg Arg	tcc Ser 70	ctg Leu	tac Tyr	aac Asn	aca Thr	gtg Val 75	gct Ala	acc Thr	ctg Leu	tac Tyr	tgt Cys 80	240
gtg Val	cac His	cag Gln	aag Lys	att Ile 85	gat Asp	gtg Val	aag Lys	gac Asp	acc Thr 90	aag Lys	gag Glu	gcc Ala	ctg Leu	gag Glu 95	aag Lys	288
att Ile	gag Glu	gag Glu	gag Glu 100	cag Gln	aac Asn	aag Lys	tcc Ser	aag Lys 105	aag Lys	aag Lys	gcc Ala	cag Gln	cag Gln 110	gct Ala	gct Ala	336
gct Ala	ggc Gly	aca Thr 115	ggc Gly	aac Asn	tcc Ser	agc Ser	cag Gln 120	gtg Val	tcc Ser	cag Gln	aac Asn	tac Tyr 125	ccc Pro	att Ile	gtg Val	384
cag Gln	aac Asn 130	ctc Leu	cag Gln	ggc	cag Gln	atg Met 135	gtg Val	cac His	cag Gln	gcc Ala	atc Ile 140	tcc Ser	ccc Pro	cgg Arg	acc Thr	432
ctg Leu 145	aat Asn	gcc Ala	tgg Trp	gtg Val	aag Lys 150	gtg Val	gtg Val	gag Glu	gag Glu	aag Lys 155	gcc Ala	ttc Phe	tcc Ser	cct Pro	gag Glu 160	480
gtg Val	atc Ile	ccc Pro	atg Met	ttc Phe 165	tct Ser	gcc Ala	ctg Leu	tct Ser	gag Glu 170	ggt Gly	gcc Ala	acc Thr	ccc Pro	cag Gln 175	gac Asp	528
ctg Leu	aac Asn	acc Thr	atg Met 180	ctg Leu	aac Asn	aca Thr	gtg Val	ggg Gly 185	ggc Gly	cat His	cag Gln	gct Ala	gcc Ala 190	atg Met	cag Gln	576
atg Met	ctg Leu	aag Lys 195	gag Glu	acc Thr	atc Ile	aat Asn	gag Glu 200	gag Glu	gct Ala	gct Ala	gag Glu	tgg Trp 205	gac Asp	agg Arg	ctg Leu	624
cat His	cct Pro 210	gtg Val	cac His	gct Ala	ggc	ccc Pro 215	att Ile	gcc Ala	ccc Pro	ggc Gly	cag Gln 220	atg Met	agg Arg	gag Glu	ccc Pro	<b>672</b>
agg Arg 225	Gly ggc	tct Ser	gac Asp	att Ile	gct Ala 230	ggc Gly	acc Thr	acc Thr	tcc Ser	acc Thr 235	ctc Leu	cag Gln	gag Glu	cag Gln	att Ile 240	720
ggc Gly	tgg Trp	atg Met	acc Thr	aac Asn 245	aac Asn	ccc Pro	ccc Pro	atc Ile	cct Pro 250	gtg Val	Gly	gaa Glu	atc Ile	tac Tyr 255	aag Lys	768

Figure 30'A"

agg Arg	tgg Trp	atc Ile	atc Ile 260	ctg Leu	ggc	ctg Leu	aac Asn	aag Lys 265	att Ile	gtg Val	agg Arg	atg Met	Tyr 270	tcc Ser	Pro	816
acc Thr	tcc Ser	atc Ile 275	ctg Leu	gac Asp	atc Ile	agg Arg	cag Gln 280	ggc Gly	ccc Pro	aag Lys	gag Glu	ccc Pro 285	ttc Phe	agg Arg	gac Asp	864
tat Tyr	gtg Val 290	gac Asp	agg Arg	ttc Phe	tac Tyr	aag Lys 295	acc Thr	ctg Leu	agg Arg	gct Ala	gag Glu 300	cag Gln	gcc Ala	tcc Ser	cag Gln	912
gag Glu 305	gtg Val	aag Lys	aac Asn	tgg Trp	atg Met 310	aca Thr	gag Glu	acc Thr	ctg Leu	ctg Leu 315	gtg Val	cag Gln	aat Asn	gcc Ala	aac Asn 320	960
cct Pro	gac Asp	tgc Cys	aag Lys	acc Thr 325	atc Ile	ctg Leu	aag Lys	gcc Ala	ctg Leu 330	ggc	ect Pro	gct Ala	gcc Ala	acc Thr 335	ctg Leu	1008
gag Glu	gag Glu	atg Met	atg Met 340	aca Thr	gcc Ala	Ç <b>ys</b> Çgc	cag Gln	ggg Gly 345	gtg Val	Gja aaa	ggc Gly	cct Pro	ggt Gly 350	cac His	aag Lys	1056
gcc Ala	agg Arg	gtg Val 355	ctg Leu	gct Ala	gag Glu	gcc Ala	atg Met 360	tcc Ser	cag Gln	gtg Val	acc Thr	aac Asn 365	tcc Ser	gcc Ala	acc Thr	1104
atc Ile	atg Met 370	atg Met	cag Gln	agg Arg	ggc Gly	aac Asn 375	ttc Phe	agg Arg	aac Asn	cag Gln	agg Arg 380	aag Lys	aca Thr	gtg Val	aag Lys	1152
tgc Cys 385	Phe	aac Asn	tgt Cys	ggc Gly	aag Lys 390	gtg Val	ggc	cac His	att Ile	gcc Ala 395	aag Lys	aac Asn	tgt Cys	agg Arg	gcc Ala 400	1200
ccc Pro	agg Arg	aag Lys	aag Lys	ggc Gly 405	tgc Cys	tgg Trp	aag Lys	tgt Cys	ggc Gly 410	aag Lys	gag Glu	ggc	cac His	cag Gln 415	atg Met	1248
aag Lys	gac Asp	tgc Cys	aat Asn 420	gag Glu	agg Arg	cag Gln	gcc Ala	aac Asn 425	ttc Phe	ctg Leu	Gly	aaa Lys	atc Ile 430	tgg Trp	ccc Pro	1296
tcc Ser	cac His	aag Lys 435	ggc Gly	agg Arg	cct Pro	ggc Gly	aac Asn 440	ttc Phe	ctc Leu	cag Gln	tcc Ser	agg Arg 445	cct Pro	gag Glu	ccc Pro	1344
aca Thr	gcc Ala 450	cct Pro	ccc Pro	gag Glu	gag Glu	tcc Ser 455	ttc Phe	agg Arg	ttť Phe	ejà âââ	gag Glu 460	gag Glu	aag Lys	acc Thr	acc Thr	1392
ccc Pro 465	agc Ser	cag Gln	aag Lys	cag Gln	gag Glu 470	ccc Pro	att Ile	gac Asp	aag Lys	gag Glu 475	ctg Leu	tac Tyr	ccc Pro	ctg Leu	gcc Ala 480	1440
tcc Ser	ctg Leu	agg Arg	tcc Ser	ctg Leu 485	ttt Phe	ggc Gly	aac Asn	gac Asp	ccc Pro 490	tcc Ser	tcc Ser	cag Gln	taa *	(SII	NO:36)	1482

Figure 30 B

Figure 31

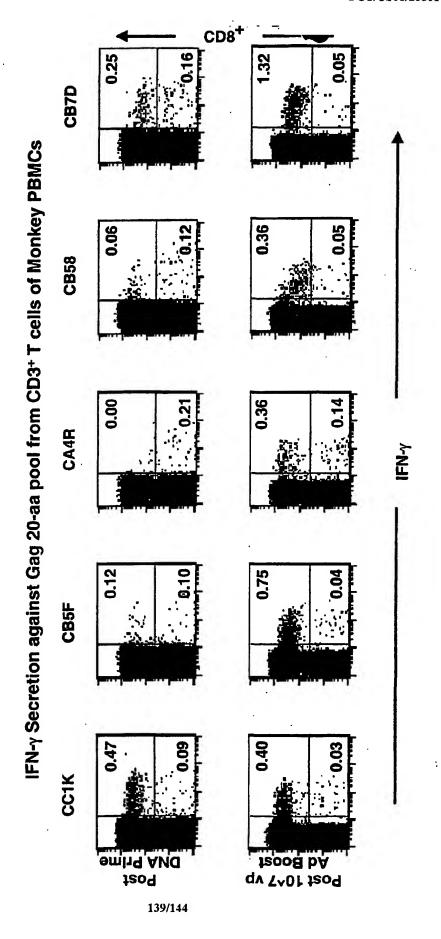
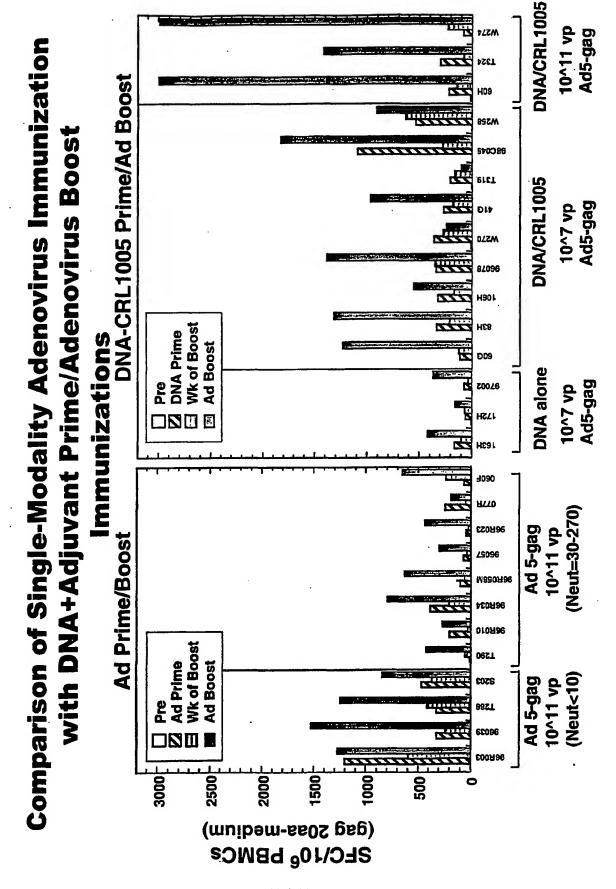


FIGURE 32



# FIGURE 33A

	GGGCTTCTGT				
CTGAGGCCTG	GTGGCAAGAA	GAAGTACAAG	CTAAAGCACA	TTGTGTGGGC	CTCCAGGGAG
	TTGCTGTGAA	· -			
-	TCCAGCCCTC				
	CCCTGTACTG				
CTGGAGAAGA	TTGAGGAGGA	GCAGAACAAG	TCCAAGAAGA	AGGCCCAGCA	GGCTGCTGCT
GGCACAGGCA	ACTCCAGCCA	GGTGTCCCAG	AACTACCCCA	TTGTGCAGAA	CCTCCAGGGC
CAGATGGTGC	ACCAGGCCAT	CTCCCCCGG	ACCCTGAATG	CCTGGGTGAA	GGTGGTGGAG
GAGAAGGCCT	TCTCCCCTGA	GGTGATCCCC	ATGTTCTCTG	CCCTGTCTGA	GGGTGCCACC
CCCCAGGACC	TGAACACCAT	GCTGAACACA	GTGGGGGGCC	ATCAGGCTGC	CATGCAGATG
CTGAAGGAGA	CCATCAATGA	GGAGGCTGCT	GAGTGGGACA	GGCTGCATCC	TGTGCACGCT
${\tt GGCCCCATTG}$	CCCCGGCCA	GATGAGGGAG	CCCAGGGGCT	CTGACATTGC	TGGCACCACC
TCCACCCTCC	AGGAGCAGAT	TGGCTGGATG	ACCAACAACC	CCCCCATCCC	TGTGGGGGAA
ATCTACAAGA	GGTGGATCAT	CCTGGGCCTG	AACAAGATTG	TGAGGATGTA	CTCCCCCACC
TCCATCCTGG	ACATCAGGCA	GGGCCCCAAG	GAGCCCTTCA	GGGACTATGT	GGACAGGTTC
TACAAGACCC	TGAGGGCTGA	GCAGGCCTCC	CAGGAGGTGA	AGAACTGGAT	GACAGAGACC
CTGCTGGTGC	AGAATGCCAA	CCCTGACTGC	AAGACCATCC	TGAAGGCCCT	GGGCCCTGCT
GCCACCCTGG	AGGAGATGAT	GACAGCCTGC	CAGGGGGTGG	GGGCCCTGG	TCACAAGGCC
AGGGTGCTGG	CTGAGGCCAT	GTCCCAGGTG	ACCAACTCCG	CCACCATCAT	GATGCAGAGG
GGCAACTTCA	GGAACCAGAG	GAAGACAGTG	AAGTGCTTCA	ACTGTGGCAA	GGTGGGCCAC
ATTGCCAAGA	ACTGTAGGGC	CCCCAGGAAG	AAGGGCTGCT	${\tt GGAAGTGTGG}$	CAAGGAGGGC
CACCAGATGA	AGGACTGCAA	TGAGAGGCAG	GCCAACTTCC	${\tt TGGGCAAAAT}$	CTGGCCCTCC
CACAAGGGCA	GGCCTGGCAA	CTTCCTCCAG	${\tt TCCAGGCCTG}$	AGCCCACAGC	CCCTCCCGAG
GAGTCCTTCA	GGTTTGGGGA	GGAGAAGACC	ACCCCCAGCC	AGAAGCAGGA	GCCCATTGAC
AAGGAGCTGT	ACCCCCTGGC	${\tt CTCCCTGAGG}$	TCCCTGTTTG	GCAACGACCC	CTCCTCCCAG
ATGGCTCCCA	TCTCCCCCAT	${\tt TGAGACTGTG}$	CCTGTGAAGC	TGAAGCCTGG	CATGGATGGC
CCCAAGGTGA	AGCAGTGGCC	CCTGACTGAG	GAGAAGATCA	AGGCCCTGGT	GGAAATCTGC
ACTGAGATGG	AGAAGGAGGG	CAAAATCTCC	AAGATTGGCC	CCGAGAACCC	CTACAACACC
CCTGTGTTTG	CCATCAAGAA	GAAGGACTCC	<b>ACCAAGTGGA</b>	${\tt GGAAGCTGGT}$	GGACTTCAGG
GAGCTGAACA	AGAGGACCCA	GGACTTCTGG	GAGGTGCAGC	TGGGCATCCC	CCACCCCGCT
GGCCTGAAGA	AGAAGAAGTC	TGTGACTGTG	CTGGCTGTGG	GGGATGCCTA	CTTCTCTGTG
CCCCTGGATG	AGGACTTCAG	GAAGTACACT	GCCTTCACCA	TCCCCTCCAT	CAACAATGAG
ACCCCTGGCA	TCAGGTACCA	GTACAATGTG	CTGCCCCAGG	GCTGGAAGGG	CTCCCCTGCC
ATCTTCCAGT	CCTCCATGAC	CAAGATCCTG	GAGCCCTTCA	GGAAGCAGAA	CCCTGACATT
GTGATCTACC	AGTACATGGC	TGCCCTGTAT	GTGGGCTCTG	ACCTGGAGAT	TGGGCAGCAC
AGGACCAAGA	TTGAGGAGCT	GAGGCAGCAC	CTGCTGAGGT	GGGGCCTGAC	CACCCCTGAC
AAGAAGCACC	AGAAGGAGCC	CCCCTTCCTG	TGGATGGGCT	ATGAGCTGCA	CCCCGACAAG
TGGACTGTGC	AGCCCATTGT	GCTGCCTGAG	AAGGACTCCT	GGACTGTGAA	TGACATCCAG
AAGCTGGTGG	GCAAGCTGAA	CTGGGCCTCC	CAAATCTACC	CTGGCATCAA	GGTGAGGCAG
CTGTGCAAGC	TGCTGAGGGG	CACCAAGGCC	CTGACTGAGG	TGATCCCCCT	GACTGAGGAG
GCTGAGCTGG	AGCTGGCTGA	GAACAGGGAG	ATCCTGAAGG	AGCCTGTGCA	TGGGGTGTAC

# FIGURE 33B

	CCAAGGACCT				
TACCAAATCT	ACCAGGAGCC	CTTCAAGAAC	CTGAAGACTG	GCAAGTATGC	CAGGATGAGG
GGGGCCCACA	CCAATGATGT	GAAGCAGCTG	ACTGAGGCTG	TGCAGAAGAT	CACCACTGAG
TCCATTGTGA	TCTGGGGCAA	GACCCCCAAG	TTCAAGCTGC	CCATCCAGAA	GGAGACCTGG
GAGACCTGGT	GGACTGAGTA	CTGGCAGGCC	ACCTGGATCC	CTGAGTGGGA	GTTTGTGAAC
ACCCCCCCC	TGGTGAAGCT	GTGGTACCAG	. CTGGAGAAGG	AGCCCATTGT	GGGGGCTGAG
ACCTTCTATG	TGGCTGGGGC	TGCCAACAGG	GAGACCAAGC	TGGGCAAGGC	TGGCTATGTG
ACCAACAGGG	GCAGGCAGAA	GGTGGTGACC	CTGACTGACA	CCACCAACCA	GAAGACTGCC
CTCCAGGCCA	TCTACCTGGC	CCTCCAGGAC	TCTGGCCTGG	AGGTGAACAT	TGTGACTGCC
TCCCAGTATG	CCCTGGGCAT	CATCCAGGCC	CAGCCTGATC	AGTCTGAGTC	TGAGCTGGTG
AACCAGATCA	TTGAGCAGCT	GATCAAGAAG	GAGAAGGTGT	ACCTGGCCTG	GGTGCCTGCC
CACAAGGGCA	TTGGGGGCAA	TGAGCAGGTG	GACAAGCTGG	TGTCTGCTGG	CATCAGGAAG
GTGCTGTTCC	TGGATGGCAT	TGACAAGGCC	CAGGATGAGC	ATGAGAAGTA	CCACTCCAAC
TGGAGGGCTA	TGGCCTCTGA	${\tt CTTCAACCTG}$	CCCCTGTGG	${\tt TGGCTAAGGA}$	GATTGTGGCC
TCCTGTGACA	AGTGCCAGCT	GAAGGGGGAG	GCCATGCATG	${\tt GGCAGGTGGA}$	CTGCTCCCCT
GGCATCTGGC	AGCTGGCCTG	CACCCACCTG	GAGGGCAAGG	TGATCCTGGT	GGCTGTGCAT
GTGGCCTCCG	GCTACATTGA	GGCTGAGGTG	ATCCCTGCTG	AGACAGGCCA	GGAGACTGCC
TACTTCCTGC	TGAAGCTGGC	TGGCAGGTGG	CCTGTGAAGA	CCATCCACAC	TGCCAATGGC
TCCAACTTCA	CTGGGGCCAC	AGTGAGGGCT	GCCTGCTGGT	${\tt GGGCTGGCAT}$	CAAGCAGGAG
TTTGGCATCC	CCTACAACCC	CCAGTCCCAG	GGGGTGGTGG	CCTCCATGAA	CAAGGAGCTG
AAGAAGATCA	TTGGGCAGGT	GAGGGACCAG	GCTGAGCACC	TGAAGACAGC	TGTGCAGATG
GCTGTGTTCA	TCCACAACTT	CAAGAGGAAG	GGGGGCATCG	GGGGCTACTC	CGCTGGGGAG
AGGATTGTGG	ACATCATTGC	CACAGACATC	CAGACCAAGG	AGCTCCAGAA	GCAGATCACC
AAGATCCAGA	ACTTCAGGGT	${\tt GTACTACAGG}$	GACTCCAGGA	ACCCCCTGTG	GAAGGGCCCT
GCCAAGCTGC	TGTGGAAGGG	GGAGGGGCT	GTGGTGATCC	AGGACAACTC	TGACATCAAG
GTGGTGCCCA	GGAGGAAGGC	CAAGATCATC	AGGGACTATG	GCAAGCAGAT	GGCTGGGGAT
GACTGTGTGG	CCTCCAGGCA	GGATGAGGAC	TAA		
SEQ ID NO:	38				

142/144

#### FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Glu Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

# FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gin Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Jle Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp SEQ ID NO: 39

# CORRECTED VERSION

# (19) World Intellectual Property Organization International Bureau





# (43) International Publication Date 21 March 2002 (21.03.2002)

# **PCT**

# (10) International Publication Number WO 02/022080 A3

(51) International Patent Classification7:

101

- (21) International Application Number: PCT/US01/28861
- (22) International Filing Date:

14 September 2001 (14.09.2001)

(25) Filing Language:

English

C12N 15/86

(26) Publication Language:

English

- (30) Priority Data: 60/233,180 15 September 2000 (15.09.2000) U
- (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): EMINI, Emilio, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). YOUIL, Rima [AU/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). BETT, Andrew, J. [CA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CHEN, Ling [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). KASLOW, David, C. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SHIVER, John, W. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CASIMIRO, Daniel, R. [PH/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (74) Common Representative: MERCK & CO., INC.; 126 Liast Lincoln Avenue, Rahway, NJ 07065-0907 (US).

- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GII, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, FT, LU, MC, NL, PT, SE, TR), OAPI patent (BP, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

#### Published:

- with international search report
- (88) Date of publication of the international search report:

  2 May 2002

Date of publication of the revised international search report: 16 January 2003

(48) Date of publication of this corrected version:

6 March 2003

(15) Information about Corrections:

see PCT Gazette No. 10/2003 of 6 March 2003, Section II Previous Corrections:

see PCT Gazette No. 03/2003 of 16 January 2003, Section II

see PCT Gazette No. 30/2002 of 25 July 2002, Section II

[Continued on next page]

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1- Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

#### TITLE OF THE INVENTION

ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

### 5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2 (serial number unassigned), filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively.

10

15

20

25

30

35

# STATEMENT REGARDING FEDERALLY-SPONSORED R&D Not Applicable

### REFERENCE TO MICROFICHE APPENDIX

Not Applicable

# FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first generation adenovirus vaccines found to exhibit enhanced growth properties and greater cellular-mediated immunity as compared to other replication-deficient vectors. The invention also relates to the associated first generation adenoviral vectors described herein, which, through the incorporation of additional 5' adenovirus sequence, enhance large scale production efficiency of the recombinant, replicationdefective adenovirus described herein. Another aspect of the instant invention is the surprising discovery that the intron A portion of the human cytomegalovirus (hCMV) promoter constitutes a region of instability in adenoviral vector constructs. Removal of this region from adenoviral expression constructs results in greatly improved vector stability. Therefore, improved vectors expressing a transgene under the control of an intron A-deleted CMV promoter constitute a further aspect of this invention. These adenoviral vectors are useful for generating recombinant adenovirus vaccines against human immunodeficiency virus (HIV). In particular, the first generation adenovirus vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide pharmaceutical products, and biologically active modifications thereof. Host administration of the recombinant, replication-deficient adenovirus vaccines described herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

# BACKGROUND OF THE INVENTION

10

15

20

25

30

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5'LTR-gag-pol-env-LTR 3'organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The gag gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the pol gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The pol gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNAse H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNAse H (RNAse, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

5

10

15

20

25

30

35

The env gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

The tat gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The rev gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

5

10

15

20

25

30

35

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8+ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8<sup>+</sup> T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4<sup>+</sup> T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

5

10

15

20

25

30

35

European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including env or gag. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see*, *e.g.*, Gräble and Hearing, 1990 J. Virol. 64(5):2047-2056; Gräble and Hearing, 1992 J. Virol. 66(2):723-731.

Larder, et al., (1987, Nature 327: 716-717) and Larder, et al., (1989, Proc. Natl. Acad. Sci. 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, Science 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, FEBS Lett. 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, J. Biol. Chem. 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, J. Virol. 69: 376-386) disclose singe and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HTV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, gag, pol and nef. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

# SUMMARY OF THE INVENTION

10

15

20

30

35

The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected 25 individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to pol modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to nef modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH<sub>2</sub>-teriminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

5

10

15

20

25

30

35

The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Poland/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replicationdefective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication -defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5'region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, a 10 more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in 15 large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use 20 in gene therapy and nucleotide-based vaccine-vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

25

30

35

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

5

10

15

20

25

30

35

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises: a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

Other aspects of this invention include a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

To this end, the present invention particularly relates to harvested recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6® cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising:

a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto, base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

5

10

15

20

25

30

35

In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

5

10

15

20

25

30

35

The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a mutlivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HTV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

5

10

15

20

30

35

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6<sup>®</sup> cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

5

10

15

20

25

30

35

It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette

comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to - highly active antiretroviral therapy -.

"first generation" vectors are characterized as being replication-defective.

They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

10

15

20

25

30

35

"s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

"Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

"Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an <u>inactivated</u> version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

5

10

15

20

25

30

35

In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or

5

10

15

20

25

30

35

"MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BgIII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IApol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt) is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the BgIII site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

"MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

"pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns and/orV1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

# BRIEF DESCRIPTION OF THE FIGURES

5

10

15

20

25

30

35

Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

5

10

15

20

25

30

35

Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

5

10

15

20

25

30

35

Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH<sub>2</sub>-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "\*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

5

10

15

20

25

30

35

Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

Figure 31 shows the intracellular γIFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti-γIFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γIFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3+ cells that were CD8+γIFN+ and CD4+γIFN+, respectively.

Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IApol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IApol fustion frame.

5

10

15

20

25

30

35

# DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus cis-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained it correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; see, Chroboczek et al., 1992 J. Virology 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6® cell line transefected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region. (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

5

10

15

20

25

30

35

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually outcompete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities in vitro when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice in vivo with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

5

10

15

20

25

30

35

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on concensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized env sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

5

10

15

20

25

30

35

A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the 5 hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at 10 least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International 15 Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an 20 amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at **25** ' the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs disclosed herein relate to open reading frames for cloning to the enhanced first 30 generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID 35 NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

5

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate 10 studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMVnef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-15 nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and 20 PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein 25 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef 30 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and 35 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

5

10

15

20

25

30

35

Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

5

10

15

20

25

30

35

The present invention also relates to application of a mono-, dual-, or trimodality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviralcontaining shuttle plasmids used in the construction of an adenovirus vector, this plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression regulatory elements, and a minimal pUC backbone; see Montgomery et al., 1993, DNA Cell Biol. 12:777-783. The pUC sequence permits high levels of plasmid production in E. coli and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

5

10

15

20

25

30

Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 pol open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine, especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein. wherein the codon usage has been optimized for expression in a mammal, especially a human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

5

10

15

20

25

30

35

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly is pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possible a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+). Potential "2+1" divalent vaccines of the present invention might be a hCMV-gagbGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (e.g.,, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficaceous adenovirus-based HIV-1 vaccine may be administered via a combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

5

10

15

20

30

35

Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon. 25 Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of E. coli most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

5

10

15

20

25

30

35

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms—a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" Advances in Pharmacology 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed supra, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6<sup>®</sup> cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6<sup>®</sup>. Both these cell lines express the adenoviral E1 gene product. PER.C6<sup>®</sup> is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6<sup>®</sup>, from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 J. Gen. Virol 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM MgCl<sub>2</sub>; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM MgCl<sub>2</sub>, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface. It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of  $1 \times 10^7$  to  $1 \times 10^{12}$  particles and preferably about  $1 \times 10^{10}$  to  $1 \times 10^{11}$  particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

5

10

15

20

25

30

35

This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

10

15

20

25

30

35

5

#### EXAMPLE 1

Removal of the Intron A Portion of the hCMV Promoter GMP grade pVIInsHIV gag was used as the starting material to amplify the hCMV promoter. PVIInsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery et al., supra for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the Msc1 site of the hCMV promoter and a 3' primer (designed to contain the BgIII recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity Taq polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with Msc1 and BgIII. This fragment was then cloned back into the original GMP grade pV1InsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following Msc1 and BgIII digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVIJnsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using BgIII digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the BgIII site. Colonies were screened using Sma1 restriction enzymes to identify clones that carried the Flgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)<sub>n</sub>, and (T)<sub>n</sub>; respectively) underlined:

<u>AATAAA</u>AGATCTTTATTTTCATTAGATCT<u>GTGTG TTGGTTTTTTGTGTG</u> (SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

Gag Expression Assay for Modified Gag Transgenes

15 EXAMPLE 2

5

10

20

25

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of

the new hCMV gag plasmid constructs have expression capacities comparable to the

original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: In vitro DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	μg gag/10e6 COS cells/5μg DNA/48 hr
HIVFL-gagPR9901 <sup>a</sup>	10.8
PVIIns-hCMV-FLgag-bGHpAb	16.6
pV1Jns-hCMV-FLgag-SPAbc	12.0

<sup>&</sup>lt;sup>a</sup> GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

10

15

20

#### EXAMPLE 3

Rodent (Balb/c) Study for Modified gag Transgenes
A rodent study was performed on the two new plasmid constructs
described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no
intron)-FLgag-SPA - in order to compare them with the construct described above
possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody
and Elispot responses (described in PCT International Application No.
PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S.
Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S.
Application Serial No. 60/148,981, filed August 13, 1999, all three applications which
are hereby incorporated by reference) were measured. The results displayed in Table
3 below, show that the new plasmid constructs behaved equivalently to the original
construct in Balb/c mice with respect to their antibody and T-cell responses at both
dosages of plasmid DNA tested, 20 μg and 200 μg.

<sup>&</sup>lt;sup>b</sup> New plasmid constructions that have the intron A portion removed from the hCMV promoter.

<sup>&</sup>lt;sup>c</sup> In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

**EXAMPLE 4** 

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA®	Dose, ug <sup>b</sup>		Anti-p24 Titers (3 Wk PD1)°		SFC/10^6 Cells (4 Wk PD1) <sup>d</sup>		
Promoter/terminator		GMT	+SE_	-SE	Media	gag197-205	p24
HIVFL-gagPR9901	200	12800	4652	3412	2(2)	129(19)	30(11)
(GMP grade)	20	5572	1574	1227	0	56(9)	25(6)
pV1Jns-hCMV-	200	11143	2831	2257	0	98(5)	12(6)
FL-gag-bGHpA	20	7352	2808	2032		73(9)	11(6)
pV1Jns-hCMV-	200	16890	5815	4326	1(1)	94(4)	26(7)
FL-gag-SPA	20	5971	5361	2825	0	85(17)	38(10)
Naïve	0	123	50	36	0	0	0

in PBS

5

15

20

Construction of the Modified Shuttle Vector - "MRKpdelE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

- (1) The left ITR region was extended to include the *Pac1* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
- 10 (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
  - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).
  - These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6<sup>®</sup> cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

bi.m. Injections into both quads, 50 µL per quad

on=10;GMT, geometric mean titer; SE, standard. error

dn=5, pooled spleens; mean of triplicate wells and standard, deviation, in parentheses;

#### EXAMPLE 5

### Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions ) and pADHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with Pac1 and BstZ1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either Cla1 linearized pAdHVO (E3- adenovector) or Cla1 linearized pAdHVE3 10 (E3+adenovector) into E. coli BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into E. coli XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction 15 digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple 20 cloning site of the original shuttle vector contained ClaI, BamHI, Xho I, EcoRV, HindIII. Sal I, and Bgl II sites. This MCS was replaced with a new MCS containing Not I. Cla I, EcoRV and Asc I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene. 25

#### **EXAMPLE 6**

## Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested

30

35

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac1* to remove the vector backbone) and subsequently labeled with [<sup>33</sup>P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

15

20

25

30

35

10

#### **EXAMPLE 7**

#### Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following coinfection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with HindIII and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with HindIII (and Pac1 to remove the vector backbone) and then labeled with [33P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

#### EXAMPLE 8

# Construction of the new shuttle vector containing modified gag transgene — "MRKpdelE1-CMV(no intron)-FLgag-bGHpA"

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with Msc1 overnight and then digested with Sfi1 for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdelE1 shuttle) was linearized by digestion with EcoRV, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdelE1 shuttle vector.

20

25

30

35

5

10

15

#### EXAMPLE 9

#### Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdelE1-CMV(no intron)-FLgag-bGHpA, was digested with Pac1. The reaction mixture was digested with BsfZ171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with Cla1 overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into E. coli BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml Terrific<sup>TM</sup> broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 μl dH<sub>2</sub>0. A 2 μl aliquot of this DNA was transformed into E. coli XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 μg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme BstEII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

#### **EXAMPLE 10**

#### Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

5

10

15

20

25

30

#### EXAMPLE 11

Virus generation of an enhanced adenoviral construct - "MRK Ad5 HIV-1gag"

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested was Pac1 to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6<sup>®</sup> cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6® cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6<sup>®</sup> cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *Hind*III and radioactively labeled with [33P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with Pac1/HindIII prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

30

5

10

15

20

25

#### EXAMPLE 12

#### Stability Analyses

5

10

15

20

25

30

35

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (in vitro gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

10

15

20

25

30

Analysis by *Hind*III digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4:
Amplification Ratios Based on AEX and QPA Analysis of Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5gag	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Flgag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

5

#### EXAMPLE 13 ·

10

Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

20

25

15

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

<sup>\*</sup> This estimation is based on the clinical lot growth characteristics at Passage 12.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32, 905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

5

10

Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

**Table 5A:** Amplification ratios determined by AEX and QPA for MRKAd5gag over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

# MRKAd5gag rep1

	Xv (10° cals/m Infaction	l), Viability (%) Harvest	Harvest Time	Cell Passage Number	Titler 10" vp/ml culture	Ther 10° vp/cell	OPA 10" TCIO <sub>se</sub> /tnt	Ratio AEX:QPA	Amplification Rate	AEX Internal Control
P4	1,49, 81%	0.58, 50%	44	46	8.7	5.9	1.72	50	470 (MOI = 125)	
PS	1,38, 93%	0.68, 47%	48	49	6.7	4.9	1.38	49	170	
P8	1.04, 94%	0.68, 77%	47	48	5.8	5.6	1.42	41	200	
P7	1.50, 84%	0.98, 61%	49.5	50	3.9	1.4	0.97	40	50	
P7	1.09, 97%	0.76, 69%	50	52	8.2	4.7	1.70	81	170	
P8	1.03, 94%	0.88, 84%	47.5	54	9.0	6.7	1.10	112	810	
PØ	0.89, 95%	0.89, 73%	47.5	56	4,4	4.9	1.03	43	175	3.12 2.84
P10	1.09, 91%	1.06, 66%	47.5	58	8.0	2.8	1.16	26	100	2.70 2.60
PII	1.19, 88%	0.88, 65%	47	60	3.6	3.0	1.15	31	110	2.70 2.70
P12	0,98, 91%	0.85, 63%	47.5	47	5.4	5.5	1.20	45	200 ·	2.68 2.60
P13	1,00, 68%	0.70, 67%	49	49	5.8	5,8	1.11	52	210	3.18 3.18
P14	1,94, 92%	0.88, 67%	48	53	8.6	4.4			160	3.28 3.27
P15	0.97, 96%	0.64, 66%	47	47	6.9	7.1			250	3.12 2.01

**Table 5B:** Amplification ratios determined by AEX and QPA for MRKHVE3 over several continuous passaging in serum free media. MRKHVE3 is the new vector backbone which does NOT carry a transgene.

## MRKHVE3

	Xv (10 collerns), Viablity (%)		Harvest Time	Cell Passage	Ther	Titer	QPA	Ratio	Amplification	AEX
	Infection	Harvest	hat	Number	10 <sup>td</sup> vp/ml culture	10° vp/cet	10° TCIDeo/ml	AEX:QPA	Ratio	Internal Contro
P4	1.10, 97%	1.28, 79%	49	54	4.1	3.8	1.70	25	300 (MOI = 125)	
P5	0.82, 89%	1.18, 77%	47	. 48	4.3	4.7	1.24	35	170	
P6	1,55,88%	1.26, 76%	49.5	50	1.2	8.0	0.58	21	30	
P6	1.09, 97%	1.11,81%	49	52	4.0	3.6	1.16	84	130	
P7	1.17, 91%	1.22, 91%	47.B	54	3.7	3.2	0.50	74	110	
P8	0.98, 88%	1.41, 83%	48	56	2.1	2.1	0,47	45	75	3.12 2.84
PS	1.20, 89%	1.28, 81%	47.5	58	0.8	0.7	0.29	28	25	2.70 2.60
P10	0.99, 82%	1.55, 85%	47	60	2.)	23	0.43	83	80	2.70 2.70
P11	1.07, 96%	1.25, 83%	48	47	2.7	2.5	0.41	66	90	2.86 2.80
P12	0.80, 91%	1.14, 80%	49,5	49	5.9	7,4	0.48	123	250	3.18 3.18
Pi3	1.95, 95%	1.14, 85%	45.5	53	5.8	3.0			110	3.28 3.27
P14	0.87, 96%	1.03, 98%	46.5	47	9.4	8.7		_	350	3.12 2.91
P15	0.87, 99%	0.87, 59%	49.5	49	5.3	6.1			218	2.78 2.52

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

**5** .

# MRKAd5gag(E3-)

	Xv (10 <sup>s</sup> cells/i infection	Harvest	Harvest Time h.p.l.	Cell Passage Number	Titer 10 <sup>49</sup> vp/ml culture	Titer 10° vp/celi	QPA 10° TCID <sub>ED</sub> Ami	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.62, 77%	1.12, 52%	47.5	48	2.0	1.2	0.92	20	100 (MO!=125)	
P\$	1.16, 92%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	. 28	100	
P6	1.09, 97%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	180	
P7	1,17, 91%	0.98, 72%	47.50	54	7.1	6.1	0,67	106	220	
P8	0.98, 88%	0.77, 48%	48	58	3.1	3.2	0.68	47	115	3.12 2.84
P9	1.20, 89%	1,03,72%	48	58	1.8	1.5	0.57	32	55	2.70 2.60
P10	0.99, 82%	0.80, 62%	46.5	60	3.2	3.2	83.0	47	115	2.70 2.70
P11	1.07, 96%	0.98, 70%	48.5	47	5.9	5.5	0.68	87	200	2.88 2.60
P12	0.80, 81%	0.67, 59%	50	49	5.1	6.4	0.72	71	230	3.18 3.18
P13	1.96, 95%	0.91, 59%	45.5	53	7.4	3.8			135	3.28 3.27
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.12 2.91
P15	0.87, 99%	0.84, 58%	49	49	4,8	5.5			196	2.78 2.52

#### **EXAMPLE 14**

#### Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

#### **EXAMPLE 15**

# Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (10<sup>7</sup> and 10<sup>9</sup> vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: In vitro analysis for gag expression in COS cells by Elisa assay.

20

25

15

5

10

Viral Vectors	µg gag/4.8x10e5 COS/10e8 parts/48hr
MRKAd5gag <sup>b</sup>	1.40
Clinical lot Ad5gag <sup>c</sup>	1.28
Research lot Ad5gag <sup>d</sup>	1.32
MCMVFL-gagbGHpA <sup>c</sup>	0.42

<sup>&</sup>lt;sup>a</sup> A<sub>260nm</sub> absorbance readings taken for viral particle determinations.

<sup>&</sup>lt;sup>b</sup> MRKAd5gag was produced in serum free conditions and purified at P5.

<sup>&</sup>lt;sup>c</sup> Clinical lot# Ad5gagFN0001

d Research Ad5FLgag lot# 6399

<sup>°</sup> mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Group	Vaccine	Dose (vp)	GMT	SE upper	SE lower
<del></del>					
1	<sup>a</sup> MRKAd5gag	10^7	25600	5877	4780
2	a	10^9	409600	94028	76473
3	hCMV FL-gag bGHpA [E3-] →	10^7	7352	2077	1620
4	nomv, z gag odr.p.v.(=o j	10^9	235253	59767	47659
5	hCMV FL-gag SPA [E3+] →	10^7	12800	9905	236
6	Now 1 E gag of A [EoI]	10^9	310419	99181	75165
7	<sup>b</sup> mCMV FL-gag bGHpA [E3+] →	10^7	44572	23504	15389
8	mom v ragag barripri (2017)	10^9	941014	239068	190636
9	<sup>c</sup> hCMV FL-gag bGHpA <b>[E3-]</b> ←	10^7	3676	934	745
10	HOMA L gag sampriles I	10^9	117627	17491	15227
11	research lot hCMV intronA FL-gag bGHpA [E3-] <-	10^6	528	262	175
12	n = 9-9 p-01-1-0 1	10^7	14703	5274	3882
13	*	10/8	58813	14942	11915
14	•	10^9	204800	53232	42250
15	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10^6	230	82	61
16		10^7	4222	3405	1138
17	•	10^8	19401	3939	3274
18	и	10^9	89144	25187	19639
19	Naïve	none	93	7	6

\*2x50 µL l.m. (guad) injections/animal

P.I.s: Youil, Chen, Casimiro Vaccination: T. Toner, Q. Su

Assay: M. Chen

5

10

#### **EXAMPLE 16**

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses,  $10^{11}$  vp and  $10^9$  vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

<sup>&</sup>lt;sup>a</sup>The structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+]  $\rightarrow$  The <u>same lot</u> of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

The same lot of mCMVFL-gagbGHpA[E3+] used in the in vitro study (Table 6) ws used here.

<sup>&</sup>lt;sup>c</sup>This construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10e7 dose from this vector is 7 fold lower then the same dose of the MRKAd5gag and 4 fold lower than the research lot.

peripheral blood assummarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with

gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk4	Wk8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
MR KAd5gaga, 10^11 vp								
97N010	<10	118	5528	11523	7062	21997	ND	51593
97N1 16	<10	62	772	1447	1562	2174	ND	20029
98X007	<10	66.	3353	6156	6845	3719	ND	24031
MR K Ad5gog, 10^9 vp								
97N120	<10	51	204	318	366	482	ND	6550
97N144	<10	18	118	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	ND	12851
Ad5gag <sup>b</sup> , Clinical Lot, 10^11 vp								
97X001	<10	_ 87	2579	4718	7174	7250	ND	69226
97N146	<10	72	3604	7380	7526	18906	ND	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
Ad5gog, Clinical Lot, 10^9 vp		<u></u>						
97N020	<10	<10	143	371	390	1821	ND	17177
97X003	<10	<10	39	93	156	596	ND	2053
98X012	<10	81	342	717	956	1558	ND	11861
MRKAd5gag (hCMV, bGHpA, E3+)								
<sup>b</sup> orlginal Actigag vector (hCMV/Intra	n A bGHp	A, E3-), lott	FN0001					
ND, not determined			L					

5

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4<sup>+</sup> T cells.

Grp #	Vaccination	Monkey ID		Wk		Wk	[a]	Wk	Tel	5 Wk		Wk		Wk
	7 =0,4,25 wks	•	Medid	Gog H <sup>b</sup>	Media	Gog H	Media	Goog H	Media	Gog H	Media	GOO!H	Media	Gog H
									_		_			
1	MRKAd5gcg	97NO10	6	89	0	395	0	1058	0	1174	3	775	4	1074
	1041 VP	97N010(CD4-)	4	38	·		3	993			0	76	0	594
		97N116	1	396	1	609	0	534	4	395	1	261	0	408
		97N116(CD4-)	[ 11	676	i .		0	593	_		0	184	0	666
		98X007	10	579	0	1304	3	2193	1	2118	3	1588	0	2113
		98X007(CD4-)	20	965			0	2675			0	1656	٥	1278
2	MRKAdago	97N120	5	275	1	249	4	141	4	119	9	206	4	219
_	10/9 VP	97N120(CD4-)	11	170			0	85			0	75	<u> </u>	219
		97N144	3	236	6	438	1	318	3	256	1	98	5	373
		97N144(CD4-)	ه	148	i	l	0	285	Į.		ND	NO	0	625
		98X008	4	388	1 1	1090	3	891	4	673	3	. 473	5	735
		98X008(CD4-)	14	696	1		0	1175	1		٥	391	4	848
3 .	Adagog dinkad lat	97X001	0	261	1	485	0	817	0	1220b	1	894	0	1858
٠.	10^11 vp	97X001(CD4-)	10	283			3	996			0	1010	0	1123
		97N146	3	150	1	465	0	339	1	1272	3	1238	3	1785
		97N146(CD4-)	6	133			0	370	,	1	0	654	l o	971
		980009	0	93	3	339	3	559	0	896	ון	384	0	1748
		98X009(CD4-)	0	73			0	333			٥	225	0	644
4	Actions dinical lat	97N020	3	30	1	101	0	8	0	36	0	26	0	41
	10/9 vp	97N020(CD4-)	10	29	Į.	i	l o	15			0	1 1	0	16
		97X003	4	68	5	184	0	18	1	38	1 4	38	6	81
		97X003(CD4-)	9	40			0	6			0	4	0	19
		98X012	5	95	3	54	1	34	0	18	0	20	1 !	121
		98XD12(CD4-)	11	70			٥	11	1		0	8	l °	41
5	Noive	96RD41	В	8	1	1	0	0	0	0	0	0	1	0
		053F	14	16	5	16,	20	14	19	15	10	15	24	١ ١

Based on either 4x10/6 or 2x10/6 cells per well (depending on spot density)

ND, not determined

5

10

15

20

"mock or no pechas control

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses in vivo even at a relatively low dose of 10^9 vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

# EXAMPLE 17 CODON OPTIMIZED HIV-1 POL AND CODON OPTIMIZED HIV-1 POL MODIFICATIONS

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

Pod of 20-capeatides overlapping by 10 caland encompassing the passequence

on that of Hxb2r, a clonal isolate of IIIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wildtype (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize in vivo mammalian expression (Lathe, 1985, J. Mol. Biol. 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294). which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

10

15

20

25

30

35

A particular embodiment of this portion of the invention comprisies codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized))" wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC

ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

	GAAATCTGCA	CTGAGATGGA	GAAGGAGGC	AAAATCTCCA	AGATTGGCCC	CGAGAACCCC
	TACAACACCC	CTGTGTTTGC	CATCAAGAAG	AAGGACTCCA	CCAAGTGGAG	GAAGCTGGTG
	GACTTCAGGG	AGCTGAACAA	GAGGACCCAG	GACTTCTGGG	AGGTGCAGCT	GGGCATCCCC
	CACCCCGCTG	GCCTGAAGAA	GAAGAAGTCT	GTGACTGTGC	TGGATGTGGG	GGATGCCTAC
5	TTCTCTGTGC	CCCTGGATGA	GGACTTCAGG	AAGTACACTG	CCTTCACCAT	CCCCTCCATC
	AACAATGAGA	CCCCTGGCAT	CAGGTACCAG	TACAATGTGC	TGCCCCAGGG	CTGGAAGGGC
	TCCCCTGCCA	TCTTCCAGTC	CTCCATGACC	AAGATCCTGG	AGCCCTTCAG	GAAGCAGAAC
	CCTGACATTG	TGATCTACCA	GTACATGGAT	GACCTGTATG	TGGGCTCTGA	CCTGGAGATT
	GGGCAGCACA	GGACCAAGAT	TGAGGAGCTG	AGGCAGCACC	TGCTGAGGTG	GGGCCTGACC
10	ACCCCTGACA	AGAAGCACCA	GAAGGAGCCC	CCCTTCCTGT	GGATGGGCTA	TGAGCTGCAC
	CCCGACAAGT	GGACTGTGCA	GCCCATTGTG	CTGCCTGAGA	AGGACTCCTG	GACTGTGAAT
	GACATCCAGA	AGCTGGTGGG	CAAGCTGAAC	TGGGCCTCCC	AAATCTACCC	TGGCATCAAG
	GTGAGGCAGC	TGTGCAAGCT	GCTGAGGGGC	ACCAAGGCCC	TGACTGAGGT	GATCCCCCTG
	ACTGAGGAGG	CTGAGCTGGA	GCTGGCTGAG	AACAGGGAGA	TCCTGAAGGA	GCCTGTGCAT
15	GGGGTGTACT	ATGACCCCTC	CAAGGACCTG	ATTGCTGAGA	TCCAGAAGCA	GGGCCAGGGC
	CAGTGGACCT	ACCAAATCTA	CCAGGAGCCC	TTCAAGAACC	TGAAGACTGG	CAAGTATGCC
	AGGATGAGGG	GGGCCCACAC	CAATGATGTG	AAGCAGCTGA	CTGAGGCTGT	GCAGAAGATC
	ACCACTGAGT	CCATTGTGAT	CTGGGGCAAG	ACCCCCAAGT	TCAAGCTGCC	CATCCAGAAG
	GAGACCTGGG	AGACCTGGTG	GACTGAGTAC	TGGCAGGCCA	CCTGGATCCC	TGAGTGGGAG
20	TTTGTGAACA	CCCCCCCT	GGTGAAGCTG	TGGTACCAGC	TGGAGAAGGA	GCCCATTGTG
	GGGGCTGAGA	CCTTCTATGT	GGATGGGGCT	GCCAACAGGG	AGACCAAGCT	GGGCAAGGCT
	GGCTATGTGA	CCAACAGGGG	CAGGCAGAAG	GTGGTGACCC	TGACTGACAC	CACCAACCAG
	AAGACTGAGC	TCCAGGCCAT	CTACCTGGCC	CTCCAGGACT	CTGGCCTGGA	GGTGAACATT
	GTGACTGACT	CCCAGTATGC	CCTGGGCATC	ATCCAGGCCC	AGCCTGATCA	GTCTGAGTCT
25	GAGCTGGTGA	ACCAGATCAT	TGAGCAGCTG	ATCAAGAAGG	AGAAGGTGTA	CCTGGCCTGG
	GTGCCTGCCC	ACAAGGGCAT	TGGGGGCAAT	GAGCAGGTGG	ACAAGCTGGT	GTCTGCTGGC
	ATCAGGAAGG	TGCTGTTCCT	GGATGGCATT	GACAAGGCCC	AGGATGAGCA	TGAGAAGTAC
	CACTCCAACT	GGAGGGCTAT	GGCCTCTGAC	TTCAACCTGC	CCCCTGTGGT	GGCTAAGGAG
	ATTGTGGCCT	CCTGTGACAA	GTGCCAGCTG	AAGGGGGAGG	CCATGCATGG	GCAGGTGGAC
30	TGCTCCCCTG	GCATCTGGCA	GCTGGACTGC	ACCCACCTGG	AGGGCAAGGT	GATCCTGGTG
	GCTGTGCATG	TGGCCTCCGG	CTACATTGAG	GCTGAGGTGA	TCCCTGCTGA	GACAGGCCAG
	GAGACTGCCT	ACTTCCTGCT	GAAGCTGGCT	GGCAGGTGGC	CTGTGAAGAC	CATCCACACT
						GGCTGGCATC
	AAGCAGGAGT	TTGGCATCCC	CTACAACCCC	CAGTCCCAGG	GGGTGGTGGA	GTCCATGAAC
35	AAGGAGCTGA	AGAAGATCAT	TGGGCAGGTG	AGGGACCAGG	CTGAGCACCI	GAAGACAGCT
						GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG

CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG

AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT

GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG

GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ

ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows: Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys 10 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly 20 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile 25 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lvs Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys 30 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu LVS Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys 10 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val 15 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr 20 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Lys Ala Lys Ile Ile Arg Asp TVT Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp 25 Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to deletion of the portion of the wild type sequence encoding the protease activity, a combination of active site residue mutations are introduced which are deleterious to HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein the construct is devoid of DNA sequences encoding any PR activity, as well as containing a mutation(s) which at least partially, and preferably substantially, abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

30

35

DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT. RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

5

10

15

20

25

~			-
. 1 . V	h	_	
10	U		

	****	aa residue	mustant an	enzyme function
	wt aa	aa residue	mutant aa	enzyme function
	Asp	112	Ala	RT
	Asp	187	Ala	RT
35	Asp	188	Ala	RT
	Asp .	445	Ala	RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

5

10

15

20

25

30

35

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC AGGATGAGGG GGGCCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG TTTGTGAACA CCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC ATCAGGAAGG TGCTGTTCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC CACTCCAACT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGAGG CCATGCATGG GCAGGTGGAC
TGCTCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC
AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGACCAGG CTGAGCACCT GAAGACAGCT
GTGCAGATGG CTGTGTTCAT CCACAACTTC AAGAGGAAGG GGGCCATCGG GGGCTACTCC
GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGGC CTCCAGGAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID
NO:3).

5

10

15

20

25

30

35

In order to produce the IA-pol-based adenoviral vaccines of the present invention, inactivation of the enzymatic functions was achieved by replacing a total of nine active site residues from the enzyme subunits with alanine side-chains. As shown in Table 1, all residues that comprise the catalytic triad of the polymerase, namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues (Larder, et al., Nature 1987, 327: 716-717; Larder, et al., 1989, Proc. Natl. Acad. Sci. 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445. Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this IA Pol construct), with each residue being substituted for an Ala residue, respectively (Davies, et al., 1991, Science 252:, 88-95; Schatz, et al., 1989, FEBS Lett. 257: 311-314; Mizrahi, et al., 1990, Nucl. Acids. Res. 18: pp. 5359-5353). HIV pol integrase function was abolished through three mutations at Asp626, Asp678 and Glu714. Again, each of these residues has been substituted with an Ala residue (Wiskerchen, et al., 1995, J. Virol. 69: 376-386; Leavitt, et al., 1993, J. Biol. Chem. 268: 2113-2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene. The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys 10 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr 15 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp 20 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala 25 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys . Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His 35 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations disclosed above may be suitable and therefore be utilized as an IA-pol-based adenoviral HIV vaccine of the present invention, either when administered alone or in a combined modality regime and/or a prime-boost regimen. For example, it may be possible to mutate only 2 of the 3 residues within the respective reverse transcriptase, RNase-H, and integrase coding regions while still abolishing these enzymatic activities. However, the IA-pol construct described above and disclosed as SEQ ID NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide such as is found in highly expressed mammalian proteins such as immunoglobulin leader peptides. Any functional leader peptide may be tested for efficacy. However, a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein the pol coding region or a portion thereof is operatively linked to a leader peptide, preferably a leader peptide from human tPA. In other words, a codon optimized HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. As noted in Figure 16A-B, a DNA vector which may be utilized to practice the present invention may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). 10 Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. 15 A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

20

25

30

35

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGT CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
GAGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT 10 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT 15 GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT 20 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA TEGECAGGTE GACTECTCC CTEGCATCTE GCAGCTEGAC TECACCCACC TEGAGGECAA GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGACAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG 25 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG 30 GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ

35 ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly

Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu 10 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp 15 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile 20 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe 25 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu 30 Thr Asp Thr Thr Asn Gln Lys Thr.Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp 35 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu · Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

15

20

25

30

35

The present invention also relates to a codon optimized HIV-1 Pol mutant contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4) which comprises a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in the above paragraphs is suitable for fusion downstream of a leader peptide, such as a leader peptide including but not limited to the human tPA leader sequence. Therefore, any such leader peptide-based HIV-1 pol mutant construct may include but is not limited to a mutated DNA molecule which effectively alters the catalytic activity of the RT, RNase and/or IN region of the expressed protein, resulting in at least substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at least one point mutation which alters the active site and catalytic activity within the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

5

10

15

20

25

30

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows: GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG CCCCGAGAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA GCTGGGCATC CCCCACCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA -GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGCCAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG GAACCCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEQ ID NO:7).

10

15

20

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu 10 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr 15 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala 20 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile 25 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu 30 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val 35 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

### **EXAMPLE 18**

## CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

10

15

20

25

30

35

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 ifrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEO ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

5

10

15

20

25

30

The nucleotide sequence of the codon optimized version of HIV-1 irfl nef gene is disclosed herein as SEQ ID NO:9, as shown herein: GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG CCGTGGCCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT ACACCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT AAAGCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby incorporated by reference. See also Figure 19A-B for a comparion of wild type vs. codon optimized nucleotides comprising the open reading frame of HIV-Nef.

The open reading frame for SEQ ID NO:9 above comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine vector. The 216 amino acid HIV-1 Nef (ifrl) protein is disclosed herein as SEQ ID 35 NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Glu Asp Glu Gly Glu Asn Asn Cys Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

15

25

30

35

HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the inner surface of the host cell plasma membrane through myristylation of Gly-2 (Franchini et al., 1986, Virology 155: 593-599). While not all possible Nef functions have been elucidated, it has become clear that correct trafficking of Nef to the inner plasma membrane promotes viral replication by altering the host intracellular environment to facilitate the early phase of the HIV-1 life cycle and by increasing the infectivity of progeny viral particles. In one aspect of the invention regarding codon-optimized, protein-modified polypeptides, the nef-encoding region of the adenovirus vector of the present invention is modified to contain a nucleotide sequence which encodes a heterologous leader peptide such that the amino terminal region of the expressed protein will contain the leader peptide. The diversity of function that typifies eukaryotic cells depends upon the structural differentiation of their membrane boundaries. To generate and maintain these structures, proteins must be transported from their site of synthesis in the endoplasmic reticulum to predetermined destinations throughout the cell. This requires that the trafficking proteins display sorting signals that are recognized by the molecular machinery responsible for route selection located at the access points to the main trafficking pathways. Sorting decisions for most proteins need to be made only once as they traverse their biosynthetic pathways since their final destination, the cellular location at which they perform their function, becomes their permanent residence. Maintenance of intracellular integrity depends in part on the selective sorting and accurate transport of proteins to their correct destinations. Defined sequence motifs exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, Cell 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, Nature Medicine 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

10

15

20

25

30

35

Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

CATGGATGCA ATGAAGAGA GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTCC TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACCCCATGTC
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCC
(SEQ ID NO:11).

The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12). Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human.

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jrfl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13,

10

15

20

25

30

GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGCCCA ACGAGGGCGA GAACAACTGC GCCGCCCACC
CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACC CGAGTACTAC AAGGACTGCT
AAAGCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

10

30

35

Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val 15 Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp 20 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His 25 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

An additional embodiment of the present invention relates to another DNA molecule encoding optimized HTV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HTV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEO ID NO:15, as shown below:

CATGGATGCA ATGAAGAGA GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG

5 GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGGGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT

10 CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTG TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCCC

15 (SEQ ID NO:15).

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro 20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr 25 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu 30 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16). An adenoviral vector of the present invention may comprise a DNA sequence, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a 35 deletion or substitution of Gly 2, a deletion of substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

20 EXAMPLE 19

10

15

25

30

35

### MRKAd5Pol Construction and Virus Rescue

Steps performed in the construction of the vectors, including the pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BgIII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 (or MRKpAdHVE3) preplasmid. The vector, similar to the original shuttle vector contains the Pac1 site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with BgI II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the BgIII site. The clones were checked for the correct orientation of the gene by using restriction enzymes DraIII/Not1. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FLpol+bGHpA(S) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)Cla1. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FLpol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

10

15

20

25

30

35

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12  $\mu$ g of pMRKAd5pol was digested with restriction enzyme Pacl (New England Biolabs) and 3.3  $\mu$ g was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc.). Pacl digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6® cells. Infected cells and media were harvested 6-10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at  $\leq$  -60°C. This pol containing recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

### **EXAMPLE 20**

### MRKAd5Nef Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector

5

10

15

20

25

30

35

MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac*1 site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*11 site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl*11 releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the

MRKpdelE1+CMVmin+BGHpA(str.) shuttle vector at the Bgl11 site. The clones were checked for correction orientation of the gene by using restriction enzyme Scal. A positive clone was isolated and named MRKpdelE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdelE1hCMVminFL-nefBGHpA(s) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdelE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6<sup>®</sup> adherent monolayer cell culture. To rescue infectious virus, 12 μg of pMRKAdnef was digested with restriction enzyme Pac1 (New England Biolabs) and 3.3 μg was transfected per 6 cm dish of PER.C6<sup>®</sup> cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech

Inc.). Pac1 digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6®cells. Infected cells and media were harvested 6-10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at ≤-60°C. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

### **EXAMPLE 21**

5

10

15

20

25

30

Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (Not I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (Bgl II)Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the Not I and the  $Bgl \Pi$  sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the El parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with Not I and Bgl II. The mCMV promoter (Not I/Bgl II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with  $Bgl \ \Pi$  and the gag reporter gene ( $Bgl \ \Pi$  fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (Asc I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (Bgl II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the Asc I and Bgl II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with Asc1 and Bgl11 to remove the hCMV-gag portion of the transgene. The mCMV promoter (Asc1/Bgl11 digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with Bgl11 and the gag reporter gene (Bgl11 fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique 35

 $Bgl \ \Pi$  site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by  $Bgl \ \Pi$  digestion.

5

10

15

### **EXAMPLE 22**

Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. Pac1 and BstZ110I digestion of each shuttle vector was performed and each specific transgene fragment containing the flanking Ad5 sequences was isolated and co-transformed with Cla I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 E. coli cells. Recombinant preplasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently prepared in large scale following transformation into XL-1 Blue E. coli cells and analyzed by restriction analysis and sequencing.

### **EXAMPLE 23**

Construction of hCMV-tpa-nef (LLAA) Adenovector

The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with BamHI, gel purified and cloned into the Bgl II site of MRKAd5CMV-bGHpA shuttle vector (Bgl II digested and calf intestinal phosphatase treated). Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following Sca I digestion. The resulting MRKAd5tpanef shuttle vector was digested with Pac I and Bst Z1101 and cloned into the E3+MRKAd5 adenovector via bacterial homologous recombination techniques.

### **EXAMPLE 24**

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c

mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol

(E3+) at either 10^7 vp and 10^9 vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively. For all rodent immunizations, the Ad5 vectors were diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl2, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50 µL aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively.

10

15

20

25

30

35

Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10^9 vp and 10^11 vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either 10^9 vp and 10^11 vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.005% polysorbate 80, pH 8.0) into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester; NY) were coated by overnight incubation with 100 μL of 1 μg/mL HIV-1 RT protein (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 uL of 1 ug/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Hunstville, AL) and incubated for 2 h with 200 μL/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was performed followed by 4-fold serial dilution. 100-μL aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100  $\mu$ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100  $\mu$ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100  $\mu$ L of 0.5M H<sub>2</sub>SO4 per well. OD<sub>492</sub> readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD<sub>492</sub> (2.5 times the background value).

Non-human primate and murine ELIspot assays - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INFγ-secreting cells from mouse spleens (Miyahira, et al.1995, J. Immunol. Methods 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at 5x10<sup>6</sup>/mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM β-ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, Current Protocols in Immunology. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100 μL/well of either 5 μg/mL purified rat anti-mouse IFN-γ IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15 ug/mL mouse anti-human IFN-γ IgG<sub>2a</sub> (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200 μL/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 µL of cell samples (4-5x10<sup>5</sup> cells per well) and 50 µL of the antigen solution were added. To the control well, 50 µL of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 ug/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4<sup>+</sup>-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8<sup>+</sup>-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8<sup>+</sup> T cell epitope) or aa81-100 (CD4<sup>+</sup>) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO<sub>2</sub>, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 μL/well of either 1.25 μg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 ug/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 μL/well 1/2500 dilution of strepavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 μL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10<sup>6</sup> cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 uL of each sample is incubated with 15 uL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN<sub>3</sub>) and 20 uL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 uL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10<sup>7</sup> vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

	TA. Transpared Darmare	,								
				An	II-RT IDG Tite	rs*	S	FC/10^6 cell	s*	
Group	Vaccine	Dosa	No. of Doses	GMT	+SE	-SE	Medlum	CD4+ peptide pool	CD8+ peptide pool	
1	MRKAd5hCMVFLpol (E3+)	10^7 vp	2 1	310419 919	301785 372	153020 265	1(1) 1(1)	75(4) 72(9)	2313(67) 533(41)	
2	MRKAd5hCMVFLpol (E3+)	10^9 vp	2	1638400 <sup>6</sup> 713155	0 528520	0 303555	2(2) 1(1)	114(9) 48(7)	2063(182) 733(89)	
3	MRKAdShCMVFLpol (E3-)	10^7 vp	2	310419 6400	385218 14013	172097 4393	0(0) 10(8)	223(7) 141(21)	2607(27) 409(28)	
4	MRKAdShCMVFLpol (E3-)	10^9 vp	2	1838400° 1241675°	0 396725	0 300661	1(1) 0(0)	180(13) 39(13)	2385(11) 833(83)	
5	Naive	none	none	57	9	7	9(2)	11(4)	10(1)	

<sup>\*</sup>GMT, geometric mean tiler of the cohort of 5 mice; SE, standard error of the gemetric mean

5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and(3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

				Aı	ti-nef lgG Tit	ers"	8	FC/10^6 cell	3,
Group	Vaccine	Dose	No. of Doses	GMT	+SE	-SE	Medium	Ba51-70 CD8+	2281-100 CD4+
1	MRKAd5hCMVFLnef (E3+)	10^7 vp	2	174 132	70 42	50 32	1(1) 0(0)	23(1) 0(0)	1(1) 0(0)
2	MRKAd5hCMVFLnef (ES+)	10 <b>^9</b> vp	2	174 132	70 42	50 32	0(0) 1(1)	61(7) 62(7)	4(2) 3(1)
3	MRKAd5mCMVFLnet (E3+)	10^7 vp	2	132 115	42 48	32 33	3(1) 3(2)	15(5) 3(2)	5(2) 4(2)
4	MRKAd5mCMVFLnef (E3+)	10 <b>^9</b> vp	2	132 132	42 42	32 32	4(2) 2(1)	83(13) 29(2)	5(1) 4(0)
5	MRKAd5mCMVtpanef(E3+)	10^7 vp	2	132 100	42 0	32 0	3(2) 3(1)	14(2) 13(4)	5(1) 10(3)
6	MRKAd5mCMVtpanel(E3+)	10^9 vp	2	230 115	170 48	98 33	3(2) 7(1)	145(29) 151(14)	4(0) 10(0)
7	Naive	none	none	152	78	52 ·	21(2)	- 18(6)	26(3)

<sup>\*</sup>GMT, geometric mean ther of the cohort of 5 mice; SE, standard error of the gemetric mean

15

Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

88

Near or at the upper limit of the serial dilution; hence, could be greater than this value

<sup>&</sup>quot;No. of Spot-forming Cells per million spiecnoytes; mean values of triplicates are reported along with standard errors in parenthesis.

No. of spot-forming cells per million splecnoyles; mean values of triplicates are reported along with standard errors in parenthesis.

peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus

10 Macaques
-------------

Vaccine (T=0,4 wks)	Monk #		Prebleed			T=4			T=7			Ta16	
7200		Mock	Pol L	Pal R	Mock	Pol L	Pol R	Mock	PolL	Pol R	Mock	Pol L	Polfi
MRKACENCMV-IApod(E3+)	99C100	1	0	Ь	1	38	31	0	52	148	0	49	715
10^11 vp	99C215	i i	2	2	10	98	249	1	109	305	22	88	250
10*11*P	99D2D1	5	5	4	6	149	85	0	40	35	0	35	18
MRKAd5hCMV-IApol(E3+)	99D212	0	2	0	4	331	114	0	58	14	0	٥	6
10°9 vp	990180	٥	4	2	0	19	192	4	38	156	5	38	108
10 , 15	99C2D1	8	5	21	6	62	62	0	18	32	۱ ا	14	65
MRKAdShCMV-I Apol(E3-)	99D239	5	2	2	20	82	172	1	66	114	9	21	40
10411 VD	99C186	4	12	6	5	120	421	2	271	489	16	B75	530
	99C084	1	8	9	8	84	464	0	14	238	1	24	264
MRKAd5hCMV-IApol(E3-)	007C	10	10	8	12	724	745	4	322	376	4	188	178
10'9 VD	CD16	l 2	0	1 1	5	474	468	0	232	212	0	101	121
10 · 4p	0011	В	6	12	10	98	110	5	60	80	8	25	34
· Nave	083Q	nd	nd	nd	nd	nd	nd	4	2	2	2	1	2

nd, not determined Reported are SFC per million PBMCs; mean of duplicate wats.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

RT ANTIBODY ASSAY TITERS IN MMU				
Vaccine/Monkey Tag	T =4	T =7	T=12	T=16
MRKAd5hCMV-IApol(E3+), 10^11 vp				
99C100	61	1999	5928	4768
99C215	81	1541	2356	2767
99D201	53	336	539	387
MRKAd5hCMV-IApol(E3+), 10^9 vp			·	
99D212	10	_40	49	68
99D180	<10	36	79	93
99C201	<10	37	71	76
MRKAd5hCMV-IApol(E3-), 10^11 vp				
99D239	44	460	1234	1015
99C186	21	· 233 ·	480	345
990084	235	2637	2858	1626
MRKAd5hCMV-IApol(E3-), 10^9 vp				
CC7C	32	175	306	235
മാഭ	20	140	273	419
Q011	15	112	149	237_

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far

5

10

15

20

25

Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus

Vaccine (T≈0,4 wks)	Monk #	Pi	ne .	T:	=4	Ţ	-7	Te	16
		Mock	Net	Mock	Nef	Mock	Nef	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CD2D	0	4	31	440	4	368	1	251
10^11 vp	CC7B	0	0	2	521	0	178	1	152
·	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CC2K	8	9	6	52	0	35	0	15
10^9 vp	CD15	5	4	30	898	2	586	0	434
•	CD16	6	1	6	1146	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D191	1	5	4	614	0	298	2	419
10^11 vp	99D144	4	6	5	434	0	1100	2	932
·	990193	1 1	2	1	58	1 1	22	0	64
MRKAd5mCMV-net(G2A,LLAA) (E3+)	99D224	1	11	14	231	1	125	0	70
10^9 vp	99D250	8	9	4	108	0	54	0	5
•	99C120	1	6	20	299	0	92	0	79
Naïve	083Q	nd	nd	18	22	4	- 5	2	1

### **EXAMPLE 25**

Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects

PBMC samples collected from two dozens of patients infected with HIV-1 in

US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping
by 10 amino acids. Four different peptide pools were tested for cross-clade
recognition, and they were either derived from a clade B-based isolate (gag H-b; nefb) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells
from these patients presumably infected with clade B HIV-1 could recognize clade C
gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated
that these T cell responses against clade C gag peptide pool were about 60% of the
clade B counterpart (Figure 24), while the T cell responses against clade C nef were
about 85% of the clade B counterpart (Figure 25). These results suggest that cellular
immune responses generated in patients infected with clade B HIV-1 can recognize
gag and nef antigens derived from clade C HIV-1. These data show that a HIV
vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapetic advantage on a global scale.

Table 15
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

subject	bleed date	gag epitope #	mock	gag H-b	gagH-c	nef-b	nef-c
		( from mapping)					
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99	4	5	1055	1080	2210	2140

10

15

20

# EXAMPLE 26 Characterization and Production of MRKAd5pol and MRKAd5nef

Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer (10 <sup>10</sup> vp/ml culture)	AEX Titer (10 <sup>4</sup> vp/cell)	Amplification Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

5 Roller Bottle Passaging - Passaging of the pol and nef constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (tritonlysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

Table		Xviable (10° cells/ml), Viabliity (%) Infection Harvest		Cell Passage Number	AHX Titer (Cell Associated) 10 <sup>10</sup> vp/ml culture		Amplification Ratio	Triton Lysis Titer  10 <sup>10</sup> vp/ml culture
hCMV-FL-nef [E3+]	pool	1.22, 85%	_	62	0.8	0.7	25	1.6
	1 2		0.99, 62% 1.10, 72%					
hCMV-FL-pol (E3+)	pool	1.42, 89%		62	4.5	3.2	115	7.0
	. 1		1.22, 70% 1.42, 74%					

5 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

15 14510		Xviable (16 Viabil Infection		Cell Passage Number	AEX Titer (Cell Associated) 10 <sup>80</sup> vp/ml culture	Titer 10 <sup>4</sup> vp/ceII	Amplification Ratio	Triton Lysis Titer  10 <sup>th</sup> vp/mi culture
hCMV-FL-acf (E3+)	Pool	1.33, 90%		66	1.0	0,8	29	2.1
	1		0.96, 70%					
	2		1.18,73%	.}	<b>'</b>			
bCMV-FL-pol [E3+]	Pool	0.90*, 90%		56	4.2	4.7	168	6.5
	1		1.18, 88%					
	2		1.04, 80%					

MRKAd5nef and MRKAd5pol Viral Production Kinetics - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of
 MRKAd5gag. PER.C6® cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

Comparison of hCMV- and mCMV-FL-nef - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate 10 produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6® cells- experiments are underway at V&CB to measure nef expression levels.

15

5

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

	ſ	Xv (10 <sup>5</sup> cells/m	l), Viability (%)	Cell Passage	AEX Titer	Titer	Amplification	Tritoa Lysis Titer
		Infection	Harvest	Number	10 <sup>10</sup> vp/ml culture	10° vp/cell	Ratio	10 <sup>10</sup> vp/ml culture
hCMV-FL-nef	Pool	1.11, 91%		60	1.5	1.4	50	2.8
(MRKAd5nef)	. 1		1.23,75%					
	2		1.34,74%		{			
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
	-1		1.49, 84%				_	
	2		1.18, 77%	<b>!</b>				

20

25

### **EXAMPLE 27**

### Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

Materials and Methods - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM Lglutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6® cells at a concentration of 0.2x106 cells/ml. Cells were grown until they reached a cell concentration of approximately 1x106 cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second 30 batch (B20010202), the same procedure as the first run was used, except the cells

were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 ℃	
DO	30%	
PH	7.30	
Agitation	150 rpm	
Sparging	None	•

Table 21: Virus source used for experiments.

10

5

Run	Batch ID	Cloned/Uncloned MRKAd5nef	MOI (vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
	B20010202-2	Cloned	280

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

15

Table 22: Virus Concentration as measured by the AEX assay

Run	Batch ID	Cloned/Uncloned	V	irus Concentration (	₱ 48hpi (1x1	10 <sup>13</sup> vp/L)
		MRKAd5nef	Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76
	B20010115-2	Uncloned	0.38	1.67	2.05	2.46
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88
	B20010202-2	Cloned	0.50	6.00	6.50	8.47

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Uncloned		Virus Concent	ration @ 48hpi	(1x10 <sup>11</sup> TU/L)	
		MRKAd5nef	Whole Broth	Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28
	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89
	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47

20

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

### EXAMPLE 28

MRKAd5HIV-1gag Boosting of DNA-Primed Animals

5

10

15

20

25

30

Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pVIJnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10e7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10e7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, CD4<sup>+</sup>-biased or CD8<sup>+</sup>-biased, and (b) boosting with the MRKAd5gag construct produced in all cases a strongly CD8<sup>+</sup>-biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific CD8<sup>+</sup> T cells.

PCT/US01/28861

-  -	Printing	Boost	Monte	T=0		Tot		T=6	9	T=10	0	Tota	4	T=24	*	1428	2	'n	97
-	T=0, 4, 8 wrks	T=28 letts		Medium	Hese	Redum	H GED	Medium	HBB	Medium	HES	Medhim	SED H	Medium	H Bei	Hedium	HOAD	Medium	8
	DNA/5 mgs	MFVCAdSgag(E3+)	CB6H	¥	¥	,	ક્ક	15		4	224	B	116	Г	83	9	958	0	316
_	PBS	4v 7v01	×8	0	0	•	5	•	48	0	89	0	28	•	32	6	1765	-	35
	(0101)		AWGG	vo	=	•	æ	n	22	m	8	8	8		89	2	686	0	395
2	DNA/6mgs +	MFRICAGSpag(E3+)	Serc	0	ŀ	-	8		E	6	82	8	280	6	22	6	959	19	1345
	CRL1005/45mgs .	10~7 vp	ž Ž	7	0	-	ĕ	0	254	•	<u>18</u>	10	\$	0	ğ	0	1915	-	560
			AWB	0	•	-	ş	•	F	*	164	•	ş	φ	8	=	8	9	241
_			OBS!	ď	ş	•	<u>ਲ</u>	•	8	0	2	6	Z,	6	251	80	1549	8	5
			AKBB	<b>"</b>	53	•	8	_	13	•	439	0	53	0	316	4	1229	9	<u>8</u>
. 69	DNA/5 mgs+	MPD(AdSpag(E3+)	AWZO	10	T	-	62	5	284	9	\$25		18	•	282	182	565	9	ş
	CRL1005/7.5 mgs + 0.6 mM BAK	10.7 10	CAAH	-	0		121	-	133	-	270	9	8	-	Ê	=	1384	9	978
			888	В	9	•	60	9	119	0	27.6	•	8	-	200	0	638	_	829
			V 685	4	6	•	8	-	16	0	139	٥	2	-	8	10	643	-	B
			C870	-	•	•	<del>2</del> 8	•	316	-	66		8	-	269	0	872	•	<u>8</u>
-	ecou	None	980201	22	0	0	0	-	•	٥	٥	٥	-	-	2		°	٥	0

### **EXAMPLE 29**

### Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the IA pol gene (consisting of RT, RNAseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNAse H and integrase (1350 amino acids; SEQ ID NO: 39).

The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IApol to a unique BamHI site. This fragment was digested with BstEII and BamHI. Construction of gag-IApol fusion was achieved via three-fragment ligation involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR product and long PstI/BamHI V1R-FLpol backbone fragment.

10

15

20

30

35

The MRKAd5-gagpol adenovirus vector was constructed using the BglII fragment of the V1R-gagpol containing the entire ORF of gag-IApol fusion gene.

### EXAMPLE 30

### Immunogenicity Studies in Non-Human Primates

Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized

HIV-1 gag, pol, gagpol, nef in rhesus macaques

5

10

Grp#	ag, pol, gagpol, nef in rhesus mac Vaccine	Monk#			T=6 wks		
	T=0, 4 wks		Mock	Gag H	Pol - 1	Pol-2	Nef
1	MRKAd5 gag	CB9V	0	15	-	•	-
i l	10^10 vp	CD19	0.	374	-	•	•
I		109H	1	843	-	•	-
2	MRKAd5 gag	99D130	1	948	-	•	•
I	10^8 vp	W277	16	324	-	•	. •
}		143H	4	595	-	-	-
3	MRKAd5 pol	CC1X	4		46	256	-
ĭ	10^10 vp	AW3W	3	-	463	550	-
ļ	12.12.12	AV43	6	-	95	1333	•
4	MRKAd5 pol	AW38	1	-	19	30	-
	10^8 vp	CC8K	0	<b>\</b> -	50	995	-
l		CC21	1	-	33	436	•
5	MRKAd5 nef	076Q	9	-	-	-	1204
l	10^10 vp	091Q	4	-			85
		083Q	0	•	•		176
6	MRKAd5 nef	00C029	1		-	-	114
1	10^8 vp	98D022	6		1 -	-	170
-		98D160	3		-	-	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D251	3	206	15	193	120
	10^10 vp each	05H	3	135	21	9	638
	·	00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D215	1	171	18	193	240
_	10/8 vp each	81H	5	73	6	14	243
	-	12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef	99D211	0	83	56	838	725
	10^10 vp each	22H	4	385	119	1194	1915
		61H	4	343	11	765	853
10	MRKAd5gagpol+MRKAd5 nef	34H	3	78	19	5	75
	10^8 vp each	48H	1	65	105	46	43
		70H	5	158	15	220	191

Indicated are numbers of spot-forming cells per million PBMCS against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10<sup>6</sup> PBMC.

### WHAT IS CLAIMED IS

10

A recombinant adenoviral vaccine vector at least partially deleted in
 E1 and devoid of E1 activity, comprising:

- a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to between from about base pair 400 to about base pair 458 of a wildtype adenovirus genome; and
- b) a gene encoding an HIV protein or immunologically relevant modification thereof.
- A vector in accordance with claim 1 comprising a packaging region corresponding to from about base pair 1 to about base pair 450 of a wildtype adenovirus genome.
- 3. A vector in accordance with claim 1 further comprising nucleotides
   15 corresponding to between from about base pair 3511 to about 3524 to about base pair
   5798 of a wildtype adenovirus genome.
  - 4. A vector in accordance with claim 3 comprising base pairs corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
- 5. A vector in accordance with claim 4 which is deleted of base pairs451-3510.
  - 6. A vector in accordance with claim 1 which is at least partially deleted in E3.
  - 7. A vector in accordance with claim 6 wherein the E3 deleted region is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

- 9. A vector in accordance with claim 1 wherein the vector comprises a gene expression cassette comprising:
  - a) a nucleic acid encoding a protein;

5

15

- b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and
  - (c) a transcription termination sequence.
- 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.
  - 11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation
  - 12. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 antiparallel orientation.
    - 13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
    - 14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.
- 20 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.
  - 16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

- 18. A cell comprising the adenoviral vector of claim 1.
- 19. Recombinant, replication-defective adenovirus particles harvested
   and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell
   line which expresses adenovirus E1 protein at complementing levels.
  - 20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.
- 21. An HIV vaccine composition of claim 20 which comprises aphysiologically acceptable carrier.
  - 22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
  - 23. A method according to claim 22 wherein the cell is a PER.C6® cell.

15

- 24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.
  - 25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

- 27. A method according to claim 24 wherein the adenovirus vaccine is
   5 preceded by an adenovirus vaccine of a different serotype.
  - 28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.
  - 29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.
- 30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.
  - 31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
- a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
  - b) a gene expression cassette comprising

- i) SEQ ID NO: 29;
- ii) a heterologous promoter operatively linked to i); and
- iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

- 33 An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

5

- 35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.
  - 37. A cell comprising the adenoviral vector of claim 30.
  - 38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell line which expresses adenovirus E1 protein at complementing levels.
  - 39. An HIV vaccine composition comprising purified adenovirus particles of claim 38.
  - 40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.
- 41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6® cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.

5

- 44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
- 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.
  - 46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
  - 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.
    - 48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.
- 49. An adenoviral vector in accordance with claim 9 wherein the gene
   20 expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.
  - 50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

5

10

- b) a gene expression cassette comprising
  - i) a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
  - ii) a heterologous promoter operatively linked to i); and
  - iii) a transcription termination sequence.
- 51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.
- 52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 20 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.
  - 56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

- 58. An HIV vaccine composition comprising purified adenovirus particles of claim 57.
  - 59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.
  - 60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

10

- 61. A method according to claim 60 wherein the cell is a PER.C6® cell.
- 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.
  - 63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
  - 64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

- 66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 5 67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.
  - 68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.
  - 69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

10

15

- a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- b) a gene expression cassette comprising
  - a nucleotide sequence selected the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15;
  - ii) a heterologous promoter operatively linked to i); and
  - iii) a transcription termination sequence.
- 70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

5

10

- 73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.
  - 75. A cell comprising the adenoviral vector of claim 68.
- 76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.
- 77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.
  - 78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.
  - 79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
  - 80. A method according to claim 79 wherein the cell is a PER.C6® cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

- 82. A method according to claim 81 which further comprises

  administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
  - 83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

10

15

- 84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
- 85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:
  - a) gag, pol, and nef, expressed independently from three individual vectors;

 b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;

- gag, pol, and nef, expressed via two vectors, one expressing a polnef fusion, and another expressing gag;
- d) gag, pol, and nef, expressed via two vectors, one expressing a gagpol fusion and another expressing nef;
- e) gag, pol and nef, expressed via two vectors, one expressing a nefgag fusion and another expressing pol;
- f) gag, pol, and nef, expressed via one vector expressing a gag-polnef fusion;
- g) gag and pol, expressed independently from two individual vectors;
- h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- i) pol and nef, expressed independently from two individual vectors;
- j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- k) nef and gag, expressed independently from two individual vectors;
- nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- m) gag and pol, expressed via one vector expressing a gag-pol fusion;

5

15

n) pol and nef, expressed via one vector expressing a pol-nef fusion; and

- o) nef and gag, expressed via one vector expressing a nef-gag fusion.
- 87. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.
  - 88. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the fused sequences have the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences.
- 89. A multivalent adenovirus vaccine composition in accordance with

  claim 86 wherein the fused sequences have the encoding nucleic acid sequences

  operatively linked to a single promoter; and the encoding nucleic acid sequences

  operatively linked by an internal ribosome entry sequence ("IRES").

### Original Adenovector Construct:

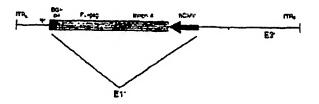


Figure 1: Original HIV-1 gag adenovector.

### Sequence of the open reading frame for FL-gag (human codon optimized)

atgggtgctagggcttctgtgctgtctggtggtgagctggacaagtgggagaagatcaggctgaggcctggtgg caagaagaagtacaagctaaagcacattglgtgggcctccagggagctggagaggtttgctgtgaaccctggc agctgaggtccctgtacaacacagtggctaccctgtactgtgtgcaccagaagattgatgtgaaggacaccaag gaggecciggagaagattgaggaggagcagaacaagtecaagaagaaggcccagcaggctgctgctgctgc acaggeaactecagecaggtgtcccagaactaccccattgtgcagaacetccagggccagatggtgcaccag gccatctccccggaccctgaatgcctgggtgaaggtggtggaggaggaggaggacttctccctgaggtgatccc catgitctctgccctgtctgagggtgccacccccaggacctgaacaccatgctgaacacagiggggggccatc aggetgecatgeagatgetgaaggagaceatcaatgaggaggetgetgagtgggacaggetgeateetgtge acgctggccccattgccccggccagatgagggagcccagggggctctgacattgctggcaccacctccaccct ccaggagcagattggctggntgaccaaccaccccccatccctgtgggggaaatctacaagaggtggatcat cecticagggactatgiggacaggttetacaagaceetgagggetgagcaggeeteccaggaggtgaagaact ggatgacagagaccctgctggtgcagaatgccaaccctgactgcaagaccatcctgaaggccctgggccctg gctgaggccatgtcccaggtgaccaactccgccaccatcatgatgcagaggggcaacttcaggaaccagag gaagacagtgaagtgcttcaactgtggcaaggtgggccacattgccaagaactgtagggcccccaggaaga agggctgctgggaagtgtggcaaggagggccaccagatgaaggactgcaatgagaggcaaggccaacttcctg ageiglaccccctggcctcctgaggtccctgtttggcaacgacccctcctcccagtaaaataaagcccgggca gat (SEQ ID NO: 29)

Figure 2

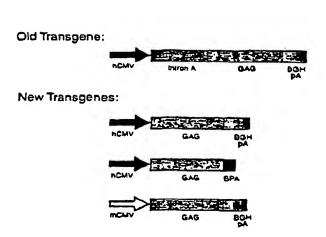


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

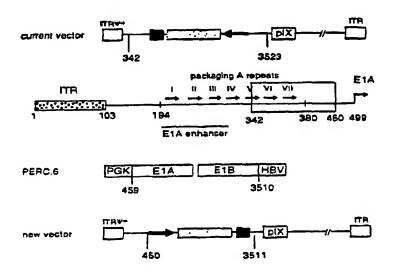


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

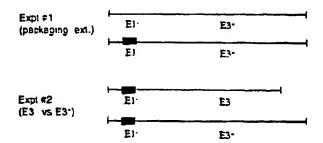


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.



Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

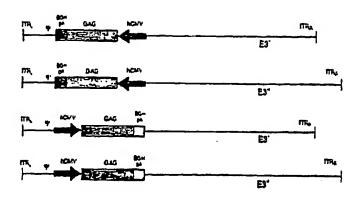


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

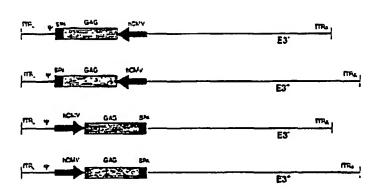


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

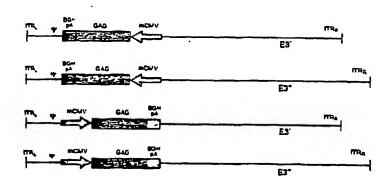


Figure 7C: mCMV-FLgag-bGHpA adenovectors constructed within the \*MRK\* backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

### Plasmid mixing expt: (orientation)

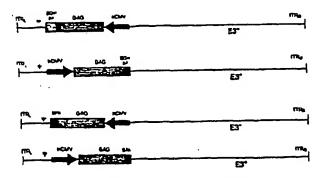


Figure 8A: Effect of transgene orientation

### Plasmid Mixing expt: (poly A signal)

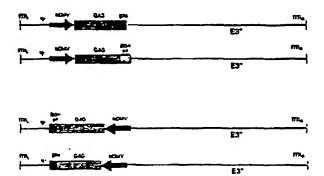


Figure 8B: Effect of polyadenylation signal

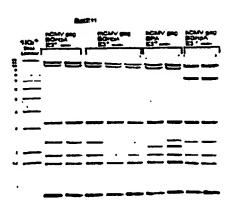


Figure 9: Viral DNA from the four Adgag candidates at P5, following BsfE11 digestion.

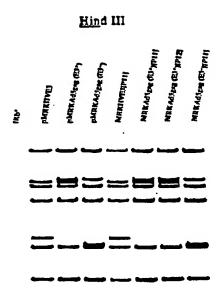


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

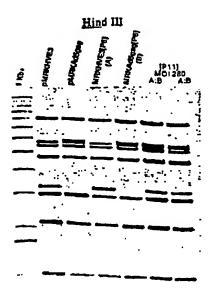


Figure 11: Viral DNA analysis (*Hin*dIII digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).

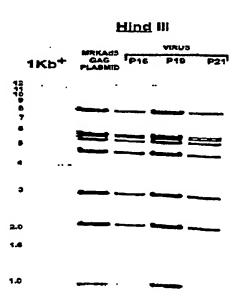
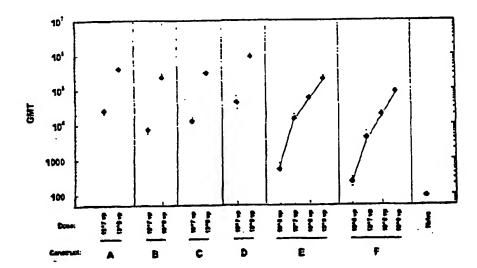


Figure 12: Viral DNA analysis by *Hind*III digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), and MRKAd5gag virus continually passaged to P16, P19 and P21(serum containing media).

Figure . Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb'c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5): (B) MRKAd5 E3\* hCMV-FLgag-bGHpA; (C) MRKAd5 E3\* hCMV-FLgag-SPA; (D) MRKAd5 E3\* mCMV-FLgag-bGHpA; (D) research Lot (293 cell-derived) of Ad5HIV-lgag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-lgag. Reponde are the geometric mean titers (GMT) for each cohort.



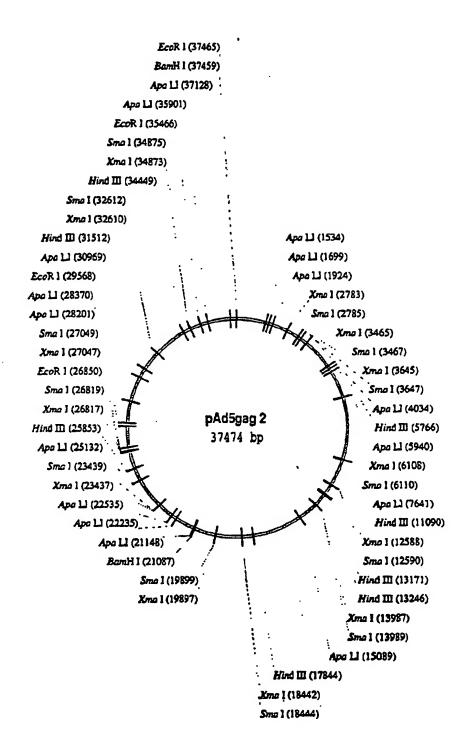


Figure 14

CCATCKGCAC בשפשמניני THYCTOCATA ATAGTATACT: CCTANOXCOTI: COCCANN'T CANCINCTICITY T CCATATCATY CCTATACTA CATTAGETTCA GTANTCAAGT AATCACCTAT **NGTACATOTA TCATGTAGA** CACACCURATA TATCATATO COCHOLOGO ACAGGGTTAR Terrecheer CAGGFATCTT ACTOCAGITA CCTACTTOGC GCATCAACCG DATTICCAND CTANAGOTTC **GCANATGGGC** COTITACCCO CTCCATAGAA ATCCAACATA TCACCTCAAT ACATCAAGTO TOTAGITICAC TCACOTTAT **OCCATITIOD** COSTINANGC CONTINCEN CCANATGCAC TACOTTOTAT ATTACGGGGT **FAATGCCCCA** בכנינטכניכנים ACTOCANANA COCUCOCUCC GTANGATTO GGACTITICAC CCTCANACTO TACCITACCT ATTACTACTOR CCCCCCCAT GROCOCOTA ATTOCONCITT TACCCTGANA GACTCACOGO CTCMGTGCCC CCCCATTGAC GOODTANCTO CICITATICAC GACAAAACTO CATTCTARAC ATCCATTGCA TATCATTAGT ACTROCOOL TOMOGETCA THYTHCACTE CTCCCAAAAG **ANACACTRICA** COGTARGTOC TATTATTATA GOCCCCCCC CTRRICTERACE GEOCYACTIAE TANACTIOCCC TPACCCACCT CATANATIZIC ATTITUACIOO ACATGACCTT ATMENDE AACAACTCCO TRATIGACIC CATTITADACE COCATIONET 2020022022 TCATTATTCA CTACTTATTA TACAACTGTA ACTAATAACT GATCAATAAT CONCINCENC TOTACTOCAN TATCCCCANA GCCATCCACG ATATTTETET AGGCCCAGG TATAAACAGA TCCCGCCCCC CONTRACTOR מבכבענכוב ACCENTINATION TIGISTACAT TCIXTUCCTA CATTANCOLA 퉏 AMMISTICGE TTTACAGCA CANATCACTT GCCAGTCTAG CGGACCTCTG TTACCOGGIG GACCGACTOS CTATTTACKE TATGCCCAGT ATACCCAPTICA ATTGGGGGGGG GPCATCHAGE TACCCCECACC GCCTGGAGAG ATCATAATKIA ACTIVITY ATTACT AACACATGTA GTANATTING ATAATATAT CCCCACCCTA CCCTGAAAGG CASCACTITICS GTTTAGTGAM CCGTCAGATC CCCCTTGCCAT CASTACATEA CLANTITUTA CCTACAACAT ATATATATA TATECHERINA CONTRACCIONAL CAMICINCAMA ATCTTCACAT ANTYRICTICAL ANTROCOTAGA ACTUALISM TCACACTCCC COCCANANCE COTTITAGG CCANANTCCS PUTCHTACTC GCCCAGTTT **GTAATGRACKS** ACTEACOGEN TCAATGCCAT **OF MICTIGUNG** CTANATICCC CATTTACCOS CCCCTTTTCC CANATICANC GETTERGITE ACTTATTANA ACACAATGAG CCCIGGTCAAA CATTGACGTC PATTITICASAT ATANANCUTA TRATESTIVICA ACTACAACGT CATTACCGCC COCMATGTAT GITCCCATAG TAACGCCAAT AGGGACTITC **GOTACCACTA** CTTTTCCCAC ACCAGACTC MOCACCET CEAGATATAT TEGTETEGAG **GCUTTACATA** TCCCTCAMO GTCAATCACC CAGTTACTOC CCATCCTCAT CAMACCGTO AMAGRICITAR TCATCTCCAA AGTACAGGTT TAATATACT ATTATATATA ההכסהאתיונה CCCCC TTCAC ATTTICKES TAMARCTATA TCANTANTT THICOTOTH CCCTATTOAC ATCCCTATTA ATCCCACTT AGAGTCCACA TTATATTOOC ANTATAACCG ATCOACTICC TACCICANGO ATTOCOUTTA COCATAACTO TAGCGATAAT TACCCTCAMA GOTCTATATA TICACTITAG TETEAGGTGT TICHTANTTA ACATCATCAA CATCATCACA **GOANGTICACA** CCTTICACTOF ANGTONNATC ANCANTEMAT TOTACTAGET GENGTAGREE COTATTACT CCCCCCACTG CCACATGROF CTTATTCTCC CAGGIGITIT GTCCACAAAA ATATOTACAT TACCCATAT ATCCCCCTATA CAMOGOTATO CANDTACOCC GITCATGCGG **GCATAATCAG** NTTCACCOTCA PACTOCAGE **FACOOTOOCA** COTOTACACA CANTARGAGG TATACATOTA CARCOGGICAC 1101 601 701 108 1001 901 501 101 101 201 5

AGGGCTTCTG TGCTGTCTGG CATHOLOMINGO CACGACTURA ACCAGAM'A CCAGATOCTC ACCACACACACC CCACCTUTIC reoremon GENCTACCAL CACCTGAGGT CCTAAACCAC ATTISTICTICOS CCTCCAAGGA TCCCCANGAC COACCITCCT AGGCTCTGAG ACCTCCARGO TYCACAGACT CTTCATKOGO TAACACGTCT TGGAGGTCCC TCCCMGACTC ATTOROGAGO TAACTCCTCC TRACATUTAE CATOGOTISCT CCCTOCIANC CAGGICCOCA CAGACOTITIO CCANTRACK CCTOGAGANG GGTTCCTCG GGACCTCTTC GANCTACCCC ATTOTOCAGA **GTACCCACGA** CENTITICETE TAACACACCC ACTICTAGATO CTCCAGCCT CHRICTARCAG CACCATTACTIC TITICARITE GRACECTACHE CACTRICCTOT MATHETECA MINNETACAA הכדנסמצכאח CITTENATORY GCCTANGRIGG CCACTCCOCA CCACCGTTCT CCFFCCCFFCFA AT:WITH AACTICACET THEMOSTURE CATTOGNACIO COGATTCCCC COLUMN TO THE PROPERTY OF THE CCACACACAT CHETYKACCA GAMPATTEAT TOXCACACAC CAGGITCHIC TICCOCCICO TCCGACGACG ACCGITATCCG TETCHOOOST AGACTCCCCA CACACCTRACT CTANCCTICC **OCTRINOGENICE** GOGAACGOTO AGAAGATCAG GCTOGAGACC TOGGACCOGA CGACCTCTOO ACCENTACT OTOTCACCOA TOCOACATOA ARKTINCTIC CCCTTGCCAC TCTTCTAGTC MODECCAGE באכנופנופנונ CAGGCGCCCG ACCCTOOCCF CACAAGTGGG CHOTHCACCC CACAGNOGCT MACCACACT DOGACATETT STECHERAG DOCTAGGTCG ACCACTCGAC THOCHORA DECTOTACAA CCCATCCAGC PROTICABOTTO 1601 1301 1501 1401 1201

Figure ISA

### PMRKAISqua HER682

1701	CACCAGGCCA	TCTCCCCCC	GACCETEMAT	CCCTOTATION		איזידידיזא ואיזידידידים		THETECHETE ARCTEANCEC	CATOTICAL	OCCUPACION IN
	GTOSTCCOST	AGAGGGGGGG	CHORDACTTA	כנאנאנינגאנד	TTT:ACC:ACT	כראנידוכנאי		TCCACTAGOO		CCANTACAGAC
1081	ACCCACGGTG	CCCCCARGAC	GACTIGHTS	ACT: NO. ACT: NO.	NITE STATES THE	CATACHER COA	CUNTERACAT	CTGAAGGAG	ACCATCAATG	ACASIANGC TO
1901	TCACTCCCAC							PUTCACATIO	THE PART INC.	CHARLEST .
	ACTEACECTS	TCCOACOTAG	מאכאנינינונינו					AGACTOTAAC	CACCOTOOTO	9Aggregatives
2001	CAGGAGCAGA	TIGGCTGGAT	GACK: ANT MAC	CCCCCCATC	AIKKKKATATA	ANTETACANG		TECTOTACET	GWCMGATT	OTTAGENTE
	Greencoter	ANCCOACCEN	CROSTICITIC	CHARRECTARGE	מאנאנבננניד	THACATOTIC	TCCACCTAGE	AGGACCCGGA	CTIGHTCTAA	CACTOCTALA
2101	ACTECECCAC	-	GACATCARSC	ACARANTECAA	משעננינונ	ACCONCTATE	TOGACAGAT	CTACANGACC	CTGAGGGCTG	ACCAROCCT
	Tologogorg	GAOGTAGGAC	CTCTACTCCC	TCCCCCCCCTT	CCTCCCCT.AAG	TCCCTGATAC	ACCTIGITOCIA	GATGTTCTCO	GACTCCCGAC	-
2201	CCAGGAGGTG	_	-	CCTOCTCSCTC	CAGAATGCTA	ACCTION	CAMBACCATC	CTGAAGGCCC	TOGRECETOC	TOCCACICTO
	OCTOCACO	TICHTOACCE	ACTOTICION	GGACGACCAC	GTCTTACKET	TOCCACTOAC	GTTCTGGTAG	CACTACCOC	ACCCCCCCOACO	ACCOURCE.
2301	CACCACATCA	•		-		CAGGGTTCTG	GCTGAGACCA	TOPCCCAGGT	GACCAACTCC	GCCACCATC.
	CHOCHCINCA	ACTOTOGGAC	<b>GGTCCCCCAC</b>	CCCCCGGGAC	CACTUTACES	GTCCCACGAC	CGACTCCOGT	ACAGGGTCCA	CTGGTTGAGG	COCTOCTACT
2401	TCATOCAGAG	_	ADCIAACCAGA	GEAAGACAGE	CAACTCCTTC	AACTRITICA	ACCTCCCCA	CATTGCCAAG	NACTOTAGGG	CCCCCCAROLIN.
	ACTACOTICE	CCCGTTGAAG	TCCTICCICT	CCTTCTGTCA	CTTCACGAAG	TRIACACCOST	TCCACCCAGT	GTACCOTIC	TTGACATCCC	<b>GOTTOTOCT P</b>
2501	GAAGGCTGC	TOCAMOTOTO	GCANGCARG	CCACCAGATG	AACCACTOCA	ATGAGAGGA	ROCCAACTIC	CTCCCCAAA	TCTGGCCCTC	CCACAACKA:
	CTTCCCCACC	ACCTICACAC	CONTROCPICE	<b>GGTGGTCTAC</b>	TTCCTGACGT	TACTOTOGE	CCRGTTCANG	GACCCONTIT	AGALLCOOOAG	GENETICEUR
2601	AGGCCTGGCA	ACTICCTCCA	<b>GTCCAGGCCT</b>	andeceance	CCCCTCCCCDA	GOAGICCTIC	AGGTTTCCC	ACCACAACAC	CACCCCAGC	CALWAGEAR
	TCCCCACCCT	TOMACCAGOT	CAGGICCOGA	CACCOCHENC	GRACAGRACT	CCTCAGGANG	TCCAMCCCC	recreation	organica merrome	CHICHTOGRA.
										Jan 1
2701	AGCCCATTGA	CANDONOCTO	TACCCCCTTGG	CENCICHOM	GICCCIGITI	OPERALTMEN	CCTCCTCCCA	GTANNITAAA	OCCCOGREGO ATCTOCTOTY	ATCTOCTOTY:
	TCGGGTAACT	OTTCCTCCAC	ATOCCCCACC	<b>GENORICIC</b>	CACACACAAA	CUSTRACTION	CCARCAGGGT	CATITITATIT	COCCCCORC	THURACCINCA
2801	CCTTCTAGT	<b>OCCADOCCATO</b>	TOPPOPURE	CCCTCCCCCG	TOCCITICOTE	DACCETGGAA	CIGTRACCACTC	CCACTOTOCCT	TTCCTAATAA	ANTERCOAN
	DOMOGRACAN	COCICOCTAG	ACANCANACG	COCAGCCCCC	ACCELACIONA	CTGGGACCTT	CCACGGTGAG	GETERCAGGA	AAGGATTATT	TTACTCCTTT
										Sphi
2901	TTOCATCOCA	TTOTCTOAGT		ADDITIONAL CHAPTETANG (RESTRANCE) (RECLARENCA	CKASTRAGASTRS	GRECARGACA	CCAACCAASA	CCATTICCGAA	GACAATAGCA	GCK: ATTCCTTCO
	AACCTAGCCF	AACAGACTCA	TCCACAGTAA	TCCACAGTAA GATAAGACCC	CCCACCCCAC	CCCGTCCTGT	COTTCCCCCT	CCTANCCCTT	CIGHTATOGT	CCCTACGACC
			Pod	3						
3001	ONATOCOGYG	<b>OCCUPATION</b>			AAATGTGTT			AGNATATATA	AGCTGGGGGT	CITATOTACE
	CCTACGCCAC	CCGRGATACC	<b>GOC-TAGCCGC</b>	OCCUCANCAC	TTTACACACC	CCCACCCAAT	TCCCACCCTT	TCTTATATAT	TCCACCCCCA	GANTACATEN
									Spirit	
3101	TITIGITATICIO	-		CCATANATACAC	CAACTICETT			ATATTTCACA	ACOCCCATCC	CCCCATOSCIC
	ANACATAGAC	ANACCTORT	בססטטטטטט	COTACTOR	CTTCACTCACA	ניזאכרידעה	NICHCTCGAG .	TATAAACTOF	TOCOCOTACO	DOCKSTACCOS
3201	COCOTCCCT	CAGAATCTCA	Transa Treas	CATHGARGE	وديدينييد	TERCETAIN	כבבבאינבאיב	TRACCTACG	AGACCOTOTO	TOCARCOCCO
	STATE OF THE PARTY	CHAPTER CAPPE	BUCKERACE	CTAACTACTA	CONTRACTOR OF THE PARTY OF THE	ACTIVITIES OF THE PERSON NAMED IN COLUMN NAMED	CACAMORMOR	S. S. C. Water S. S. Williams		ACTURE STORY

tique 15B

# PHRKAdSgag MER682

figure 15c

# PMRKAdSgag MER682.

4901	CONTROCTED	Acarterer	a contract to							
	CCACCCCAAC	-	_		CCCACACACC	G-ACCEPTICAGE	CERTAIN	Grander	ACCACACTAT	CACCACACACAC
5001	TCCGCGGCGT	GOCCCTINGC	_		Articles Control A		THEMENORER	Transference		Opposite Contract
	AGGCGCCGCA	CCGCGGACCG	_	_	Tecarcages	_	ACCTUTANA	ACTCCCCCAT	CTCGAACCCC	CECTUTION
5101	CCGATTCCGG	GONGTAGGGA	TCCCCCCC		CACTOCAL					**************************************
	<b>GCCTAAGGCC</b>	CCTCATCCGT	ACCCCCCCCC	-	CTGCCAGAGC			GAGACCGGCA	AGCCCCAGTT	Tringentry.
									110111078	1071
5201	<b>PCCCCCATGC</b>	-	GTTICTTACC	TCTOSTTTECC	ATVIANZONOT	GICCACACIC	GCTGACGAAA	AGOCHETECG	TOTCCCCGTA TACACIACTTY	TACAC:ACTT
	AGGGGGTACG	~	CAAAGAATGG	MGACCANARG	TACTITIZATEA	CARGITACGAG	CCACTOCTTT	TCCGACAGGC	ACAGGGGCAT	ATCICTGAM:
		Khol								•
5301	AGAGGCCTGT		•	Techechen	ATAGMACTC	GACCACTCT	CACACAAACC	CYCOCOTOCA	DOCCAGGACO	AAGGAGGCTA
	TCTCCGGACA	_	ACANGGEREC	NGCAGGAGCA	TATCTTRIAG	CC. TOOTGAGA	CICIOTITICS	OAGCCCAGGF	CCGGTCGTCC	THECTECGAT
5401	ACTOGGAGGG	Ξ	THEFCACTA	GOOGGICCAC	TUTCHECONG	MCTCAAGAC	ACATOTOCOC	CICITICOCCA	TCARGGARGO	TOATTASTE
	TCACCCTCCC	CATCOCCAGE	AACACOTCAT	CCCCCAGGTG	MACHINACTEC	CACACTTCTB	TOTACAGOOG	GAGAAGCCGF	AGTICCTICC	ACTACCAM
5501	Gracototac	-	-	TGANGGOGGG	CTATAAAACC	<b>GOOTTOGGGGC</b>	OCUTTODEC	TCACTCTCT	CCCCATCCCT	GICTOCOAGO
	CARCCACATC	COCHOCACTO	GCCCACAAGG	ACTICCCCCC	GATATTTEC	CCCACCCCCC	CCCMRCAGG	ACTGAGAGAA	DOCOTAGEDA	CAGACCTEC
5601	OCCAGCYCT	GOOGTGAGTA	_	ANAGCOCCCA	TEMCFECTIC	GCTAAGATTG	TCAGTTTCCA	AAAACGAGGA	DOATTIGATA	TICACCINGO.
	COGICGACAA	CCCCACTCAT	GALOCCIACACT	TITCGCCCGT	ACTIONAGACG	CGATTCTAAC	ACTCANAGGT	THICKLE	CCTAAACTAT	AACTOCIACCTI
							Hamelli	1		
5701	CCCCCCCTCAT	OCCTITIONOO	осститала втоссосля	CCANCICAGE	AGAMAGACA	ATCHTERIT	TGTCAAGCTF	GETGECUME	GACCCGTAGA	CARRESTATION
•	DOCCCACTA	COMMACTIC	CACCGGCGTA	OCTACACCAG	Territor	TAGANAMACA	ACAGITICGNA	CCACCOFFTO	CTGGGCATCT	CCCCCAACCT
	•				Paret					
5801	CADCAACTTO		<b>OCHOXOTTIO</b>	GETTERIORGO	CCATORCCC	<b>actecitions</b>	CGCSATGTTT	AGCTGCACGT	ATTEGEORGE	AACCCACCCA.
	OTCOTTOAAC	COCTACCTOS	CONCCCARAC	CAMANCAGE	מכדאמככסכם	CCACATARCCO	GCCCTACAAA	TCCMCOTGCA	TANGCGCGCG	TIGGOTGGCJ
5901	CATTCOOGAA	AGACOGTGGT	<b>GCOCTCOTCO</b>	GOCACCAGGE	CCACINITICA	ACCIRCUCTEG	TOCAGOGTGA	CANCOTCAAC	OCTOOTOOCT	ACCICICON
	GINACCCTT	Terroccacca	COCCAGCAGC	CCGTGGTCCA	COTOCOCAST	TOXCOCONC	ACCITOCONCT	Grecogrid	COACCACCOA	TOCAGAGG
1009	GFACOCOCTC	<b>GTTGGTCCAG</b>	CAGAGGCGCC	COCCCTIVATO	CGARCAGAAT	GALGATAGAG	GGTCTAGCTO	CONCREGACE	occocata	CONCCACTOR
	CATCCCCCAB	CHACCHOSTIC	פוכונכפככם	GCGGGGAAACAC	OCTUMENTA	CHICKLANCEC	CCAGATCGAC	GCAGAGCAGG	CCCCCAGAC	<b>GCAO</b> GTTTC;CA
6101	AAAGACCCCG	GOCAGCAGGC	OCCOUNTCOAA	<b>STASTCTATC</b>	THECHTECT	GCMGTCTAG	מתכדותכדונו	CATCCCCCCC	COCCAACCE	<b>GCCCTCTTAT</b>
	TTICTOGGGC	cconcorceo	COCCCACCT	CATCAGATAG	AACGTAGGAA	CULTICAGATE	GCTASACGACG	פואבמכמכבכ ו	OCCEPTICACO (	COCCAGCATA
6201	COULTICAGE	GOOGNECCCA	<b>TOCCATORIO</b>	TOCOTTACCO	CHINCKACCTA	CATCCCCCAA	ATCTCCTANA	CCTAGAGGGG	CTCTCTCAGT A	ATTECHANAT
	CCCAACTCAC	CCCCTOOOOT	ACCOTACCC	ACCEMENTOC	<b>OCCTOCATAT</b>	GTACCOCCTT	TACAGCATTT	GCATICTICCCC (	CAGAGACTCA .	TARGGTTCTA
6301	ATOTAGOOTA	<b>OCATICITICEA</b>	CCCCCATATIC	TEXACTACAC	GTANTCHTAT	Anthronoca	MACCONCIONS	CACCTCCCCA (	CCGAGGITGC	TALIGRACIAN:
	TACATCCCAT	COTAGNAGOT	OGCGCCTACO	ACCOCCCCTG	CATTAIX:ATA	TCANGCACGC	TCCCTCOCTC (	CTCCAGCCC+ (	OCCUCANCO 1	ATTACCCTACCT
6401	CHOCHCHOCH	COGNIGACTA	TCTCCCTCAA	GATGACATGT	CALIFORNIA	ATATOTHES	ACCIONATIONS	ACCITICANCE 1	TOTOTOTO	GAGACTTACT:
	GACGNEACCH	<b>OCCTACTOM</b>	AGACCOCACTT	CTACCCTACA	CTCAACCTAC	TATACCAACC	TOCCARCETTC	TOCANCTICE I	ACCCCAGACA (	CTCTCX:ATCF:

Figure 150

## PMRKAdSgag MER682

										100
6501	acofcacoca	COANGOAGOC	GTACKARITECO	CACACICITAGE .	אנישינגשיני י					TECTIONISA
	COCAGTGCGT	GCTTCCTCCG	CATCUTCARC	פניבובנישכע ז	ACTIVATIVE AG	כניגאליי אניונים	ACCITICACAT	CCCCCCTCAT O	CAGGTCCCAA	MOGMACTACT
1023	STATE AT A CHE	_			THE STATE OF THE S	AMTHTMOTO (	CONCERNICA	GENCTUTIO	ATCCACAAACC	כמונטענבוי.
1000	ACAGTATGAA					איזידאיזאניני	CCAGAMAGOT	CATGAGAACC	TAGCCTTTGG	מניענינינינציא:
6703	CENTRAL PROPERTY					AKKATOTOTE '	THETACKEST	AGCGCCTATO	ccrococooc	CTTCCOMP
	CCTTOCCATT					TEXTENDEDA	AAGATCACICA	1CGCGCA7AC	COACOCCCCO	CAAGGCCTV":
6801	CACCTOTOCO		GOTOTOCCTO	ACCARCACT	TENZETACTE		TCAGTGTCGT		CTCCTCCCAG	ACCARARAT
1	CHCCACACCC		CCACARGGAC	TYGTACTGAA	ACTICATIVAC	CATAMOTHE	ACTEACAGEA	GCGTAGGCGG	GACGAGGGTC	<b>TCGTTTTTCA</b>
6901	CCONGCGCTT	THOCHACGO	GCATTTGGCA	TOCCOANGOT	מאכאזיימתו	AACACTATCT	אי נונמנוכני אי נונמנוכני	<b>NCCCATABAG</b>	TIGCORTICA	TGCGGAAGG
•	GUCACCCGAA	ANACCTTOCO	CCTAMCCGT	CCCGCTTCCA	CTRITARICANC	TRECATAGA	MARGOCOCOC	TCCCTATTIC	AACOCACACT	ACCCCTTCCT:
7001	TCCCTGCACC	: TCGGAACOOT	TOTTAATTAC	CTOCACOCCI			CHICATCHIC	TOCCCCACAA	TOTALANGITIC	CANGAAGCG
	AGGGCCGTOG	ADCCTIOCCA	ACANTTAATG	GACCCCCCCCC	TCGTGCTAGA	GCALITITICOG	CANCTINCANC	ACCGGCTCTT	ACATTICANG	מחכיחליה:
7101	GOGATOCCCT	r TONTOGANGO	CATTITION	AGTICCTOGE	ACCITICACITIC	TTCACKXXXCAG	CHICACCICGE	GCTCTGAAAG	GACCCAGTCT	CCANGATICAG
1	CCCTACGGGA	_	GTTAAAAAT	TCANGGAGCA	TECALITICANS	ANGHOCCOTO	GACTEDOOCA	CCACACTITIC	CCCCCTCACA	COTTCTACTI:
1201	CATHOGAAGE	CACCAATICAG	CTCCACAGGT	CACAGGCCAT	TAGEATTEC	ARRITEGICAC	GAMAGMICCT	ANCTOCCGA	CCTATOCCCA	THEFTICAGE
	CCARCCTTCO			MOCCCOGTA	ATCGTUNGG	TECHCENCE	CFTTCCNXCA	TTTGACCGCT	GCATACCOOP	MAMANGACC
1901	CONTRACTAG			TCCCAGCC	TCCCATCCA	CETTERCOCKEC	TAGGREGATO	OCCOCACTOR	CTAGAGGCTC	Archecocca
	CACTACATIC			AAGGOTCGCC	ACCUTAGENT	CYANACGCCG	ATCCAGAGCG	CCCCGTCAGT	GATCTCCGAG	TAGAGGCGGC
1401	A CONTROL OF THE PARTY	_		TEXTECCOAL	AGGCCCCCAT	CUANCTATAG	GICTCIACAT	CGTAGGTGAC	AAAGAGACGC	TCGGTGCGAA
406/	THE REPORT	-		ACCAROCETT		CONTICATATE	CACACATGTA	OCATCCACTO	TTICTICTOCO	AGCCACGCT
	,	Assistantes Control of	The Control of the Co	ATTICOUR A	ATTENCENCE	TYSCTATTGA	TETESTRADA	<b>GTAGAAGTCC</b>	CTCCCACCCC	CCGAACACTC
106/	CATCLIACL			CCCCCTTGCT	TANCCINCT	ACCGATAACT	ACACCACTET	CATCTTCAGG	GACCICTACCC	OCCITICADA
	בוארתרורפת					CTACATOCTIC	CACCACCTTO	ACCTGACGAC	CGCGCACANO	GAAGCAGAGT
1097	פוסכוספכוז				Acceptance	CATGTAGGAC	GTCCTCCAAC	TOCACTOCTG	TOCACTUCTE GEOCOTOFFIC	CTTCGTCTCA
	CACGACCGAA	A ACAITIIIS	בשרמרפונאו	פארראורפר				2	10	
	A CANADA PARALLE	- Carrierance	TATESTAL .	CACTICATION	CITICIACTIC	CARCINCTION	CCTTCACCGT	CHARGETOCTIC	CTURCTUCTC CAUGODAGTE	ACCOPTICATY:
3	COLLEGE			CCGACCACCA	CAACATCAAG	CCCACCIAACA	CATANCTA KATA	GACCGACGAG	CTCCCCTCAA	TRECARCETA
-		_		AGATOTECOC	COCCORCOGIT	CYSTACICTIVIA	TEMENACATE	GCCCAGATGO	GAGCTOTCCA	TOCTCTOOM
108/	CONCENSION			TCTACAGGCG	CHECKEGOCCA	<b>GCCTCGAACT</b>	ACTOTTOTAG	COCCITCTACC	CICCOACACAT	ACCAGACCTC
				Ē						•
1006		C. CHENESCHICALISTS	CONTRACTOR IN	CTUSCACATIT		ACCITCOCATA GACKASCICAG ISCOCKABCT AGATCCAGGT GATACCTAAT	GOCOCGGGCT	AGATCCAGGT	GATACCTAAT	Trechagate
106		_	COCCUTCOAG	GACGTCCAAA		CHIRCCCAGIC	CHIECCEAGIC CCGCGCCCGA	TCTAGGTCCA	CTATOGATTA	MOGICCCC
						Krist	_1			
1000			CONTRACTOR ASSESSMENT AND CONTRACTOR CONTRACTOR CONTRACTOR CONTRACTOR AND CONTRACTOR CON	AGGCCGCATC	ניבנטכנישעונ	מארידאריאחיוא הי יאריברייאכם הסכואכידססכ כטכססמאביום	ני אנונכנאכם	COCCUCAGOOC	COCOCCAGIO	TCCTTCCATT
8001	ACCARCCACC		GEOCOGRACIA ECGANCIATIC TECAGOGIAG TAXANCETTO ETGATEXUAT TACREGOLIGE CEGECOACETO GEOCEGEORICA	TECAGEGIAG	ממטטטטענ	CTGATGCUAF	מאכנאכטכנוסכ	CCCCACCCO	<b>OCCCCCCCAC</b>	ACCOUNTETAL.

### PMRKAdSqag MRR682

8101	ATCCATCTAA	ANGCOGTOAC	<b>GCGGGCGAAG</b>	ריבניימאמיד	Nasasaku	בכישיאכבנייור	CACCAGACAC	GCCAGGGCA	COTCONICCE	GCGCGCGCAX;
	TACGTAGATT	Treaceacte	COCCURTING	CHARTICAL		GRECTINGGICG	נאגיני <b>ו</b> כי וככ	ccorcccor	CACCCATOD	CCCCCCCCC
8201	ASSASCTURE	OCTOCOCCC				GITTANTETY		accretector	CANCACCACC	מנוכנומשוניא
	TCCTCCACCA	CONCOCOCOCO	_	COCHECICA	מיזמימנים:	CAACTAGACE	N TTANAMICG	CCONGACCCA	<b>centraction</b>	CCGCCCACT
8301	CCTTCANCCT	CAAAGAGAGT	TECHENIANT	CAATTTCCCT	CHESTITICALS	כאנושיניניאנאינ	CHANANTETE	CTYSCACGITCT	CCTCAGTTGP	CTTCMTAGG.
	COAACHTOCA	CTTACTCTCA	AGCTGTCTTA		GTTAMACICA CARTAACITCC	כניניההאהרכה	CCTTTTAGAG	GACOTOCAGA	CCACTCAACA	GAACTATCC:
					101					
8401	<b>GATCTCOOCC</b>	ATGAACTOCT	CONTCINE	CHCCTACKAGA	בחניניחית:מה חביויניוניהוני	כנאנשנשבשב	CACOCTROCC	GCGAGGTCGT	TOGRANTOCO	COCCUTGAL "
	CTAGAGCCGG	TACTTGACGA	OCTACACAAA	GARTACETET	NUMBER	GCCCAGCGAG	CTICCACCCC	CCCTCCAGCA	ACCITITACGE	CCCFTFACTC:
8501	TOCCHOANGO	COTTORACICC		CASACGCIANC	なずからみのころの		CCATCGCTATG	COCCECATGAC	CACCTIGCOCG	AGATTCACE "
	ACOCACATICC	GCAACTCCGG	AGGGACCAAG	GICTGCGCCA	ACA'R: TGGTG	CHASCIACIANASC	CCTAGCCCCC	CICACOTACTO	OTGGACGCGC	TCTAACTCG.
8601	CCACGTOCCO	GOCGAAGACO	GOCGANGACO GCOTAGITTIC	GCACATOCTO	AAACAGGTAAG	THEMERICAN	TGGCGGGGGGG	TYCTOCCACO	AAGAAGTACA	TANCCCAR!
	COTOCACOCC	COCCUTCTOC	CCCCTTCTGC CCCATCAAAG	COTCCGCGAC	TITICICCATC	AACTCCCACC	ACCECCACAC	AAGACGGTGC	ITCITCATOR	ATTGGGTCKA:
		₩ <b>ξ</b>	Ecoffy							
8701	recedents	CATTCOTTCA	GATTEOTISA TATECECCAA	<b>GCCCTCAAGG</b>	CCCTCCATCS	הכדרהדאמא	GTCCACOGCG	ANGTTONAM	ACTOSCAGIT	GCCCCCCAC
	ACCOPTICCAC	CTRACCAACT	CTRACCAACT ATACCCCTT	CCCCAGTTCC	OCCOMMENCE	GRAGCATCTT	CAGGTGCCGC	TICANCITIT	TOACCCTCAA	סבכתכספכות
8801	ACCOUNTANCY	CCTCCTCCAG	ANGACCOATO	AGCTCGGCGA	CAGTOTCOCG	CACCTORNIC	TCANAGGCTA	CAGGGGGCCTC	TICTACTICT	TCAATCTCE:
	TOCCULTICA	CCACCACCTC	THETECETAC	TCGAGCCGCT	GTCACAGCGC	GTGGAGCGC	AGETTCCCAT	GTCCCCCGGAG	ANGANGA	ACTTAGAGGA
									Sp	3
8901	CTTCCATAG	<b>GOCCTCCCCT</b>	refrement	CTOCCOCCC	TOCOCCACE	GGGACACAGC	GGCGACGACG	<b>GCOCYCCOOO</b>	AGGCCCOTCCA	CAMACCOCTC
	GAAGGTATTC	CCCCCACCCCA	AGANGANGAN	GACCOCCOCC	νισεισεισε	CCCTGTGCCG	COMPACTOC	COCOTOGCCC	TOCKCOACT	GTTREAMS
9001	DATEATETEE	CCCCCCCCAC	OCCCCATOR	CHECKITISACE	CTCCCCCCCT	TUTCHCCCCC	GCCACTIVA	AAGACGCCGC	CCONCATGIC	CCGGTTATGG
	CTACTRONGO	OCCCCCCTC	CCCCCTACCA	GAGCCACTOC	COCOCCURKIN	AGAGGCCCC	CCCCACCACC	TTCTGCGGCG	OCCUPTACAG	<b>OCCCANTACK</b>
9101	GTTOGCOOGO	<b>COCHOCCATO</b>	COCCAGGGAT	ACCCCCTAA	CGANTEATER	CARCANTING	TUTTANCTA	CICCOCCOCC	CACCODACCTO	ACCCARTER
	CARCCOCCC	CCCACOGFAC	<b>GCCGTCCCTA</b>	TOCCGCCATT	CCTACCTAGA	GTTCTTAACA	ACACATCCAT	GAGGCGGCGG	CTCCCTGGAC	TCGCTCAGG
			XOrd							
9201	CATCUACCOG	ATCOGAMAC	CTCTCGATIMA	ACCUSTCTAA	CCAGTCACAG	TCCCAACGTA	GACTGAGGAG	COTGOCOGIC	ООСУВСОВОЕ	OCCOPTODITION:
	GTAGCTOGCC	TAGCCTTTG	GAGAGCTCTT	TCCGCAGATT	GGTCAGTGTC	ACCGTTCCAT	CCCACTCGTG	GCACCGCCCG	CCGICGCCCG	CCCCCAGCCC
							Sati			
9301	ortorricio	GCCGAGGTGC	TOCTGATGAT	<b>STANTTANG</b>	TARKEDSTRIFT	TRAGACTGCG	GATGGTLTCAC	AGAAGCACCA	TOTOCHOOD	Techacettee
	CARCANAGAC	COCCHICAGO	ACCACTACTA	CATTANTITIC	ATCEXXICAGA	ACTICTOCCOC	CTACCAGCTG	TCTTCGTGGT	ACAGGAACCC	AGGCCGGGACG
9401	TOANTECCEA	OOCOOTCOOC	CATTACCCCAG	<b>OCTIONAL</b>	CACATICITICS	CACAMICTERS	TAGENGETT	CCATGACCT	TTCTACCOCC	ACTICITICE
	ACTTACOCCT	COCCYGCCG	GTACCOCOCTC	CGANGCAAAA	CTCTACCCOC	CHECAGANAC	ATCATCACAA	COTACTEGGA	AACATOCCO	TGANGNAGA.
9501	CHECTHOCHE	TTOTOCTOCA	TCTCTTCCAT	CTANCACHOC	بعديمينهند		CTANTITUTE	CCCTCTTCCT	CCCATGCOTO	TGACCCCGA
	CACCAACCAC	AACAGGACGT	AGAGANCOTA	GATMX.GACG	כניאכניאכניטכ	CTCAAACTTG	CATCCACCCC	CCCACAATCA	COGENCIACIO	ACTEGORET P
9601	acceptance	COCTCAACCA	GARCTAGGIC		CCCTTTTTTTT		CHICACCTOC	GTCAGCAGTAG	ACTORANGE	ATCCATETICY.
	COOCAGTAG	CCCACTACCT	CCCCATCCAG	ccacrorrac	<b>GCCACCCCAT</b>	TATACCCCORC	CACCTCCACG	CACTCCCATC	TGACCTTCAG	TAGGTACAGO

		County Boundary	The Water State of the State of	CHAPTER SPECIAL	ACTIVITIES OF AT	NACTORACTAG 7	TTAACGGCTCT	CATTACCCG	CTGCGAGAGC	TCCCTICTACC
10/6	ACRAMIALISE	MINITERIAL						CCACTORSCC	CACCACTCTCG	ACCCACATOR:
	TOTTICGCCA	CCATACCCC CCACAMCTAC		CM ATRIACO						
		ě	_						1	
1000	ACTIONS	GFAAGTCTC	GAGTCAAATA	בנים אים בנים בי אחרים בינים		ACCUSTANCE CATARCECAC		CAMMAGIGC	OGCONCOCCT	COCOCITAGAG
1006				CCATCACA	נינובעניאיניני	TREPRESENTIA CCATAGOSTO		<b>GITTTICACO</b>	CCCCCCCCAA	CCCCANCIC
						Fort	_1			
1000		Acarataca	Casa the Casa	CASTGAGATOT	TECAACATAA	CARCCATEATA TECESTAGATO	TCCCTACATO	TACCTOGACA	<b>ECCADOTICAT</b>	מכבססבטובם
1066	Trong and a	TCCCACCGGC	CCCCAGGCCC		AGGITGIATT	CCCCTACTAT ARXCATCTAC	ARXCATCTAC	ATCCACCTGT	AGGICCACTA	כמכנמכנמכ
	OFFICEROCAGE	٠.	GHUTANGONUG	COULTECAGA	TOTAL	CTTCAAAAAG	POCTCUATED	TOGGGACGCT	CTOOCCOOTC	Argerarders.
10001	CACCACCTCC		CAGCGCCTTGC	CCCMCCTCT	ACARGOGAC	GCCGITTITIC	ACCUAGGTACC	ACCCCTGCGA	CACCOGCCAG	TCCGCGCGC;
		Xbal					:			
10101	AATCOTTOAC	<b>OCTUTAGACC</b>	<b>STOCKNANGS</b>	AGACCCTGTA	ACCORPATALL	CTRICGROCT	CTGGTGGATA	ANTICOCANO		ביסיובייייייייייייייייייייייייייייייייי
	TTACCACTO		CACOTITICC	TCTCGGACAT	TOGCCCGTGA	CHACACACTA	GACCACCTAT	TTAGGGTTC	CCATAGTALL	GCC 10C 10c
10201	GOOTTCOAGE	CCCOTATCCO	OCCURCUIC	<b>GTRIATECATE</b>	CONTINUES	CCCCCTIVITY	ANCCCARRIE	TOCCACCICA	GACMACCARR	GAGIGCICCI
	CCCAAGCTCG	_	COCCAGGOOT	CACTAGGTAC	GCCANTGOCK	CHICKICACAGG	TIVERSTICCAC	ACCCTOCAGE	CHEMICCCC	CTCACCACCA
10101	THE PERSON		COCCOCTOCT	GCGCTAGCTT	TTTTCTCCAC	PRESCONARIO	CHACGTAAGC	CONTRODUTO	GAMACCANA	GCATTANGT:
	ANACCGRACIO		CCCCCCACCA	COCCATCOM	ANNECOSTO	ACCOGGGGGG	GICCCATTCG	CCAATCCGAC	CTITICOCTITI	CCTAATICAL
10701		_	CASSITIATITIE	CCAAGGGTTG	ACTICACOCA	CCCCCOGNIC	CACTUTUCGA		TOCOGCGAAC	
40501	CONGCONG	-	CCCANTRALA	CONTECCAAC	TCACCCCCT	<b>GOTTOCCANS</b>	CTCAGAGCCT	OCCOOCCIO	ACOCCOCTTO	
•	CONTRACTOR OF THE PARTY OF THE	-	CHARTERABA	THEFT	AACAGOCAGO	ACCCCCTITT	TICCTUTICC	CAGATOCATO	COGTOCTOCO	-
10501		_	GCGANCETT	ANGENORCE	THENCOTOC	TCGGGGAAAA	MCGANAAGO	<b>OTCTACGTAG</b>	CCCACCACCC	CONCINCIO
,			POPULATION	CACATACAGA	CATCACACAC	Arceneers	CCTCCTACCG	CCTCAGGAGG	<b>OCCGACATOC</b>	
10601			The state of the s	CHEGEGGIET	GINCOLOGO	TYCCARCTES	GENGENTAGE	<b>OCASTCCTCC</b>	CCCCTOTAGO	-
	SCOOLS OF THE PARTY OF THE PART				CONTRACTAC	CTRCACTTO	ACTAGGCCCA	0000010000	-	-
10701	COCCAGCAGA	TOURNAME OF THE PARTY OF THE PA		רושומשרכנו	CACCCUTCATO	_	Trefreecent	CCCCCCAGACCCCC	<b>OCCONTICENC</b>	פכםעמשטאר:
			Secretary British	WINT BUTTER	CACCCCCTACC	TOCCITOTA	GAACCTGFFFF	COCTABOCOCO	AGGGAGAGA	_
10801	TOMOCOGONC	CCAMOSONG	Try But 18	ACTATICCOCA	CTCCGCATGC		CTTCSCACABA	<b>accertoococ</b>	receneration	coortecte
	אבונפרנסו	Service of the servic			ATCCCTCAA	TECCARCAG	THECHOCICO	AGGAGGACTT		CCCCCAACCC
10601	TACCCCCTAG	CHITCARGOT			TACCOCACT	AGCGCTCGCC	AACGACGCGC	TCCTCCTGAA	ACTICIOGOCTO	COCOUNTOGO
										-
100.1	A STATE OF S	. Craceratera	CACGIGGGG	CCCCCCACCT	<b>RETANCURCA</b>	TACCIACACA	CECTGANCCA			
10011		-	CHESCACCIOCC		CCATTGGCGT	ATCATACAT	CCC ACTICATI	CCTCTMTTG	AMGRETATI	
					GCACTCATT	ATCTCTCACA	CTTTRATAMOC		-	
111101	CCACCIOCOF	F ACCUMENT				TAGACACCCT	CANACATTCO	COCCUACCTICO	-	
					ACANTIGARIT	ATTICACIONAL	מכמבדמבלו	_	-	
11201	GAOTACCOCO					TANGTECKTA	COCCACCAT	TOTATICATET	. מססכובכבפ	מכפעכבפשכם

		-								
11301			AMCATECTO CAGAGITATING		CHTT-WATTE	ARTHRACTY			TATTCCATCC	HEAGCCTOSS
	ADCTANACTA	TITOTACCAC	CICICOTATE	אנא:אני אנייני	ביים אינו דינור צרי	THE TATACHTCAN	TEST RESEARCE		ATAMGGTACG	ANTICIALACITY
11401	CAAGETTEAC		TATACCATAC		CULINIAGA	אראזארונידאא	CATCCACATA		CCATCCCCCT	CAARTTRICT .
	CTTCAAAATO	COCOCCHICT	ATATCKETATO	COGNATOCAL	מטידאתיתים	TITLYCATIT	כדאית דכמים	ANGATUTACO	COTACCOCOA	כבבעיכעכני י
11501	ACCTITGAGGG		CGITTATOCAC	MCGMATGCA	TOURNAME	CATGAISCOTTS	איככטניימיב		CGACCCCCAAG	CIVIATUXCACA
	TODANCTOCC	TOCTOGACCC	CCANATARED	TRICICOCGE	MOTOTICE TRICKS	GCM:TCCCCAC	Tracecand	COCTCGAGTC	GCTGGCGCTC	GACTACORUT
11601	OCCTOCINADO	OCCURRENT	COCATORISCA	CCCCCCATAG	NUMBERCHAR	TCCTACTTRG	Acococococ	TGACCTGCGC	TOGGCCCCCAA	GCCCAACTCGF
	CCCACCTTTC	CCGGGACCGA	CCGNCCCG	CRECKKETATE	TUTCCGCCTC	ACCATCAAAC	TOCACCCACG	ACTOCACOCO	ACCCCCCCCTT	CONCINCOCO:
11701	CCTGGAGGCA	OCTOGGGCCG	GACCTORGCT	RACTION TRACTA	בבניניניניניני	CTCCCAACCT	CONCRECENC	GACCINATATE	ACCAGGGACOA	TCACTACGAG
	GCACCTCCCT	CONCECCOOC	CHOCHOCOCO	CCCCCACCGT	<b>BOXILLIANCE</b>	GACCGTTGCA	OCCCCCCCAC	CTCCTTATAC	recreetoer	ACTICATOCTIC
								·	P313	
11801	CCAGAGGACG	OCCAGTACTA	AGCGCATGATG	TITCHGATCA	CATCATCAA	GALFICANCIAS	ACCERACISE	GCGGGCGGCG	CTGCAGADCC	ACCCUTCCO :
	GENETICCIOC	COCTCATOAT	TCGCCACTAC	AMGACTAGT	CTACTACGIT	CTOSCULTURES	TOGGCCCATCA	CCCCCCCCCCC	GACCICTOO	TODOCAGGC
11901	CCTTAACTCC	ACCORCGACT	<b>accection</b>	CATGGACCIBC	ATCATCTCCC	TRACTIFICACIO	CAATCCTFAC	<b>acomocooc</b>	AGCAGCCGCA	GGCC/MCCr31
	<b>CCAATTCACO</b>	TOCCTOCTOA	CCCCCCCTCCA	GTACCTGGCG	TAGTACAGEG	ACTIGACCECC	<b>GTTAGGM:10</b>	CCCANGCCCC	TCGTCGGCGT	COOCULOCC:
		•					P. C.			
12001	CTCTCCCCAA	TTCTOGARGE	OCHOPICCCO	OCCCOCOCA	ACCCCACTICA	CRACANDOTO	C'HOGCGATCG TANADOCOCT	_	GCCCGAAAAC	AGGCCATC
	CAGAGGCGTT	. AAGACCTTCO	CCACCAGGC	COCCCCCCTT	TOGGGTGCGT	<b>ACTOMICCAC</b>	GACCGCTAGC	ATTTCCCCCA	CCCCCTTTG	TCCCGGTAR
12101	GGCCCGACGA	GOCCOGCCTO	GTCTACGACO	CHICKETTICA	CCCCCTCCCT	CCTTACAACA	GCGCAMICE	GCAGACCAAC	CTOSACCOOC	TOOTOOGGGA
	CCOOCTOCT		CAGATGCTGC	GCGACGAAGT	COCCICCACCEA	CCANTISTAGE	CRECKTTROCA	ceremonno	GACCTGGCCG	ACCACCCCC"
12201	TOTOCOCOAG		AGCGTGAGCG	CRECEARGEAR	CAGGGCAACC	TOCOCTOCAT	GGTTCACACTA	AACOCCTICC	TGAGFACACA	OCCCOCCAN'
	ACACGCCCTC		-		grecentes	ACCECENGETA	CCANCOTCIAT	TTGCGGAAGG	ACTCATGTOR	COCCICCITY
10261	Contraction		THE LANGE AND	S. C. VICTOR	CACTORICANT	AATTENTACT	CACACACTEC	AAACTUAGGE	GTACCAGTCT	COCCCACACT
10091						STATE OF THE REAL PROPERTY.				Craciation 19
	כאכספכפכבכ	CIGICCICCE	CATOTOCCTO	CATGICGING AMERICACICGE	כוגיעכפכרייש	TIMECALIGN	CITION CALL			received that
12401	ATTITUCEA			NGACCGTARA	CCTMARCCAR		ACTTOCAMO			CCACAGGGGG
	TAMAMAGGT	CTAMEATER	GTICCOGACG	TUTOSCATIT	CCACTCGTTC	CCANAGETITE	TRIMACETICAC	כמשניאככככב	CACOCCCOAG	Gricicaer
12501	CCCCCCCCACC	GIGICIANCE	TOCTGACOCC	CAACTORCIAC	CTGTTTT TOC	TYACTANTAGE	GUTTETTE	GACAGTOGCA		<b>GGACACATAC</b>
	<b>GOCOCOCTGO</b>	CACAGATOGA	ACGACTGCG	GFTGAGCGCG	GACAACGACG	ACGATTATCG	CTRANACTURE	CTCTCACCGT	CCCACAGGGC	CCTOTOTATO
12601	CTAGGTCACT	TOCHOACACT	OTACCGCGNG	<b>GCCATAGATC</b>	AGGINGTATES	CHALLACTAT	NITTICCARG	AGATTACAAG	TOTCAGCCGC	COCCULACION
1	CATCCAGTGA				TCCCCCTACA	CCTIXITCGITA	Transcorce	TCTAATOTTC	<b>ACAGTCOGCG</b>	COCCOVCCCC
								- 4	Presi	
12701	AGGAGGACAC	COCCACCTO	CACKCANCE	TANACTACCT	CATTUMETANCE	CTARTARCAGA	AGATOCOCTO	OPPOCACACT TRANCACCO		ACCARTAGET
	recreeters				CCACTICATION	CHECKETET	TUTACKATAG	CACCTGTCA	ANTITION	PECTECTALY;
12801	CATTITIOCOC		AGAGCTATCAG	CCTTAACCTG	ATTACORYS	CH KETTAACINIC	CARCATOCALG	CTRRACATGA CCROGORIAN		CATCHALALCTE
	GTANAACGCG			CCAATTCCAC	TACKTATAC	CCCATTRACGG	GTCCACACTURE	GACKTROTACT	COCOCOCOTT	GTACCTTOOS:
										•

Figure 15H

## PHRKAd5449 MERGR2

12901	OCCATOTATO	CCTCAMCCT	OCCUPERATE	AACTURITAA	TYXIACTA	מבעשנושנו	שרנארנהדיא	ACCCCCMOTA	TTTCACCAAT	CCCATCTICA
	CCCTACATAC	GCAGTITICOC	CRECAMITAG	TTXXXXXXTT	ACT TR:ATC:AA	THE STATES	CONTRACTOR T	THANKTOAT	ANACTOCTTA	CENTACAACT
13001	ACCCRCACTO	OCTACCOCC	CCTCATTATA		אדיגיאייידא	רויזימאיזיזא			GACATAGACG	ACACACATOTT
	Trace of the	CGATGGCGG	GUALCANAIA	אייונאמרניני אייונאמרניני	TAMERITERS	raza perent	ירטי.דאבר.דאמי י	HINGER COLUMN	CTOTATCTGC	TOTOCCACAA
13101	TECCEGGA	CCOCAGACCC	TOCTACACT	GIMCHAILE	GAPCARACAG	MARKARIA	GCGAAAGGAA AGCTTCCCCA		GOCCAAGCAG	CTIGICCGAT
	AAGGGGGTFF	000000000	ACCATCTCAA	CGITICICOCC	والإنصلاميان	TUCKTOCKTICA	CUCTITICCTT TCGANGGCGT		CCGOTTCGTC GAACAGGCT.	GANCAGGCT.
					I linelli					
13201	CTACCCCCTC	COCCCCCCC		OTCANATOCT AGTACCCCAT	TTCCANATT GATAGGGTCT	GATAGEOTET	CTTACCARCA	CTCCCACCAC	CCCCCCCCCC	CTCCTOTACC:
	DATCCCCCAC	GCCGGGGGCGC		CAGTETACGA TEATCOGGTA	AAGGTTCC:AA	CTATCCCAGA	CANTGOTCGT	GACCOTGOTO	accapacaca	CACCACCC
			PSA	_						
13301	ADDADDAGTA	CCTAMACAAC	-	TEGETISETISE ANCERCAGING	CGANANANC	כומככובטמם	CATTROCCA	CAACGOGATA	CACAGCCTAG	TOGACANIAT
	<b>TCCTCCTCAT</b>	COATHOTIC	AGEGACGACG	TOGGOGINGO	Centime	GACCAGAGICC	GTAMGREET	GTTGCCCTAT	CTCTCGGATC	ACCTI:TTCTA
13401	<b>CAGTACATOS</b>	ANGACCTACG	COCAGGAGGA	CARGGACGTG	CCAMPACTEGE	CONCENTRACION	COSTEGICAA	AGGCACGACC	GTCAGCGGGG	rendencina
	CTCATCTACC	TRCTGCATGC	GOGICCTOT	GITCCCITGCAC	ממוניתיתית	CCARGONAGEO	GOCAGCAGTT	TCCGTGCTGG	CASTCOCCC	AGACCACACC
13501	CACCACCATO	ACTOGGCAGA	CGACAGCAGC	GICCTCCATT	TRICESACRETAG	TEXTACCES	TTTGCCCCACC	TYCGCCCCAG	CCTCCCCCACA	ATCTITAM
	CTCCTGCTAC	TOAGCCOTCT	<b>GCTGTCGTCG</b>	CACCIACCTAA	ACCCTCCC+TC	ACCETTEGGC	AAACCCCTUS		COACCCCTCT	TACANAATT
13601	ANNMANAAA	GCATGATGCA	AMTANAM	CTCACCAAGG	CCATTACACC	GACCTITICAT	TETCTIVITAL	TCCCCTTANT	ATOCOGOGO	COCCCATGTA
	etheriters:	COTACTACGE	TITATITIE	GAGTRIOTTCC	CASTACASATAS	CHYCCAACCA	ANNGAACATA	ACTOGRATICA	TACCACCACAC	OCCULTACAT
13701	TOAGGAAGGT	כבוכבובכבו	CCFACCIACAG	TOTOGRANIC	CATTANETAG	TRANSCOCKAS	OCTOBOTTE	CCCTTCGATO	CTCCCCTOGA	CCCCCCTTT
	ACTCCTTCCA	CCACCACCCA	GGATGCTCTC	ACACCACTCG	CCCCCCCCC	ACCOCCOCCG	CCACCCAAGA	COGRACIETAC	CAGGGGGACCT	GOCCOCIA
		E C								
13801	<b><i><u>ofocortects</u></i></b>	GGTACCTGC	<b>GCCTACCOX</b>	GREAGNANCA	CEATCOUTTA	CICTIPACTIC	<b>GCACCCL:TAT</b>	TOPACACCAC	CCGTOTGTAC	CTGGTGCCACA
	CACCOCACCCC	CCATCGACGC	COGATOCCCC	CCCTCTTIGE	CCTAGGCAAT	GATACTCAAC	CCTCCCCCATA	ACCTURATE	CCCACACATO	GACCACCTOT
13901	ACANGTCANC	GGATCTGGCA	TCCCTFAACT	ACCANARGA	CCACACCAAC	TTICTGACCA	CONTRATTO		TACABECEGO	GGCAGGCAAG
	TOTTCAGTIG	CCTACACCOT	NOCCACITION	TREFFTREET	GETERCOTTO	AAAGACHEST	CCCAGTAAGT	THEFT	ATCTCISCCC	CCCICCOLD.
14001	CACACACACC	ATCARTCTTO	ACCACCOSTC	<b>GCACTCASCASC</b>	CKICTACCTVIA	AAACCATCCT	CCATACTAAC	ATGCCAAATG	TOAACOAGTT	CARTITIACC
	<b>GROTOTICTOO</b>	TAGITAGAAC	TOCTOCCCAG	נישפאכבכבפ	CCCICTOGACT	TTTCCTACTA	CGTATACHTG	TACOGITITAC	ACTTGCTCAA	GTACAMTEG
14101	ANTARGITTA	AGGCGCGGGT	GATCCTOTCO	COCTROCCTA	CTAAGGACAA	TYANTHICAN	CTGAAATACG	AGTOGGTOTA	OTTCACGCTG	CCCCANARACA
	TTATTCAAAT	TECECOCCEA	CTRCCACAGC	OCCUNCOCAT	CATTCCTV:TT	AGTCCACY-TC	GACTTTATGC	TCACCCACCT	CAAGTGCGAC	accreceer
				ŧ	Puri			•		
14201	ACTACTCCGA	GACCATCACC	ATAGACCTTA		CATCHICAGA	_	AACTCXXCAG		_	CHECONCATEOR
	TOATOMOOCT	CHOOFACTOO	TATCTIZCAAT	MCTACHTICO .	CTAGE:ACC:TC	CHEATCANIT	THEACCOSTC	-	_	CACHETAGE
14301	COTAMOTH	GACACCCGCA	ACTTCAGACT		دندسدسين		CCCTTTTTA	_	_	TCCAGACATC
	CCATTICAAA	CTGTGGGCGT	TOMOTOTOR	CCCTAAAI.TII	באיווטאיצצא		COMPUCCTAT		TTCCCANCGT	ACCICIONA
14401	ATTTOCTOC	CACCATOCOG	_	ACCCACACACAC	מהכדייתאא	CHICFRESAC	APETTACAMGE			TTRACATCA
	TAMANCACG	פוככדאכמככ	CCACCTRIANG	TOXILOROG	COCACTORITY	GANCANCCUG	TAGGETTEG	CCCTTCCCAA	פטרכיוככנפ	MUTELIAN

Figure 15I

14501	CCTACGATGA	TCTGGAGGGF	CCTAACATTC	CCCCACTICAT	CHEATGREAC	CATTACTORS	CHARCTINIAN	AGATGACACC	GAACAGGGCG	COCCITOCOCO
	<b>CCATTRICTACT</b>	AGACCTOCCA	CCATTICTANG	GOCCITUACAA	הכדאהאנדיזם	CCSTATICSTIFIC	<b>CCTCCANCTT</b>	TCTACTGTGG	CTRITCCCGC	CCCCYCCGCC
14601	AGOLGGCAGC	AACAGCAGTG	OCANTION CO.	CHANGACAAC	PERCANCIONS:	CACATTTACKE	AATGCAGGG	CACCHETER	TCAACCATCA	TOCCATTORY:
	11111111		C. C							
14701	OCCURCACOL	-	COCTCACGAG	MIXINGUM	אימיניו יונאאאין	MATTERITAM	פונידע מיברוא דרכ	CCCCTCCCCCA	ACCCOAGOTIC	GACAACCT
	CCGCTGTGGA	AACOGTGTGC	CCCACTCCTC	TREGOCULAC	reconcinen	TCCCCCT	CTACCCCATG	GOCGACGCOT	TOGOCTCCAG	CTCTTCGGW
										Parameter .
14801	ADMANANCE	<b>CCTCATCAAA</b>	CCCCTGACAG	NOTACACCAA	CANACICANT	TACAACCTAA	TAMECAATOA	CAGCACCTTC	ACCCAGTACC	CCACI-TCT-TA
	<b>TCTTCTTTGG</b>	CCACTAGITIT	<b>GGGGACTIGTC</b>	TCCTOTCOTT	CTITICOUNCA	ATCITICALATT	AFFECTIACT	GTCGTGGAAG	TOCOTCATOO	COTTCHACCAT
	<u>\$</u>									•
14901	CCFTCCATAC	ANCTINCOGCO	ACCURCACAC	COCIANTECES	TEATTACK	TYCTTHICAC	TCCTOACCTA	ACCTGGGGGT	COCHOCAGO	CTACTCSTCY:
	CCANCOTATO	TTGATGCCGC	TECONOTETO	CCCTTAGREC	ACTACTOR	ACCAMANGE	ARBACTIZAT	TOGACGCCGA	accrearcea	GATGACCARIC
15001	TTGCCAGACA	TOATOCAROA	CCCCCHCACC	THOUSEPICA	CONTRACTOR	CARCANCTITY	CCCSTRACTOG	GCCCCGAGCF	OFFICECCOTO	CACTRICANGA
	AACOGICIGE	ACTACOTTCT	OCCUCACTOO	AAGGCGAGGT	GCGCCCTCTA	GTCCTTGAAA	GRECACEACE	COCCOCTCOA	CAACGOGCAC	OTCAGGTTCT
										Astd
15101	OCTICIACIA	COACCAGGC	GTCTACTCCC	AACTCATCTO	CCAGTTTACC	TETETRACCE	ACCTIONTICAL	TCCCTTTCCC	GACAACCAGA	THISCOCIATI
	COAMGATGIT	OCTOOTCCGG	CAGATGACO	THEADTAGGE	<b>GCTCANATGG</b>	AGAGACTGGG	TOCACARGIT	AGCGAAAGGG	CICTIOORCE	AAAACCUCCH"
	Đ,									
15201	CCCCCCAGCC	CCCACCATCA	CCACCGTCAG	TGAAAAGOTT	CCTACTCTCA	CAGATCACCC	CACCCTACCG	CTGCGCAACA	CCATCGGAGG	AGRECAGETIA
	<b>GOOCGOTCOC</b>	COCTOCTACT	<b>CONTRACTOR</b>	ACTITICCAA	GCACCAGAGT	GTCTAGTGCC	CTYCCATCCC	CACCCONTCT	CCTAGCCTCC	TCACCTCC(T
15301	<b>GTGACCATTA</b>	CTGACGCCAG	ACCCCCCACC	TUCCCCTACO	TTTACAACAC	CCTCCCATA	סוכיוכטככטכ	GCCTCCTATC	CAGCCCCACT	TITTOMOCIA
	CACTGGTANT	DACTECOGRE	TOCOOCCIOO	ACCIONOCATOR	AMTOTICCO	GRACCCGTAT	CAGAGCCGCG	COCAGGIATAG	CTCGGCGTGA	AAAACTICITT
15401	<b>GCATOTCCAT</b>	CCTTATATCO	CCCAGCANTA	ACACAIRCETG	המאכיהכטכ	TTCCCANGCA	AGATICITATION	COCCOCCAAG	ANGCOCTCCO	ACCAACACC
	COTACAGGTA	GGAATATAGC	<b>COCTCOTTAT</b>	TOTOTOCOAC	CCCCCACCCC	AACCONTCIT	TCTACAURIC	<b>OCCCCOSTIC</b>	TTCGCGAGGC	TOCTIVITORY:
15501	ACTOCOCCTO	COCOOOCACT	ACCOCORDIC	CTOCOCCCC	CACAAACTECTS	OCCUPANTAGE	מנטכאכניאכב	GTCGATGACG	CCATCOACOC	<b>GOTCHTTCA</b>
	TCACCCCCAC	<b>GCGCCCGTGA</b>	TOCCOCOCO	GACCTICGCCAC	<b>ORGITTICCOC</b>	COCCUENCE	CCCCTCCTCC	CAGCTACTOC	<b>GGTAGCTGCG</b>	CCACCACCT
15601	GACCCCCCA	ACTACACGCC	CACOCCCCCCA	CCAGAGATICA	CACTOGACGC	COCCAPTICAG	<b>ACT. GTG/TT/C</b>	GCTGAGCCCG	GCGCTATOCT	ANNATONICA
	CTCCCCCCCO7	TOATOTOCOU	GTGCTACCOOT	GUTCACAGGT	GICACCTGCG	CCAGTANGIC	TOTICACCACG	CCCCHOOOCC	CCCCATACGA	<b>דידיאכידיכ</b> יי
15701	GACCOCCACAG	GCGCGTAGCA	COTOCCACC	CCCCCCGACC	CHACACTRICC	מבכנאוניצנו	טשיכשיכשיב	CCTCCCTTAAC	COCOCACOTO	GCACCTARICG
	CTOCCOCCTC	COCGCATCGT	<b>GCACCOOTOS</b>	במככמסכיונים	CCCCTGACGG	COCOTTOCOC	פכבטכנטכנפ	GCACCAATTO	CCCCTCCAG	CONGCCOOC
		SHI								
15801	ACCIOCOCOCC	ATGCCCCCC	CTCCIAAGCCT	GACCOCONOT	ATTROPCACTO	TERCENCECE	GTCCAGGCGA	CCAGCGGCCG	CCGCAGCAGC	COCONCCATI
	TOCCOOCTOG	TACCCCCCCC	GAGCTTCCGA	CCCCCCCCCC	TAACAGTIAC	ACTAGOCATIC	CARGITECT	<b>ACTICOCCOR</b>	<b>GCCGTCGTCG</b>	acacantaa
15901	AGTOCTATICA	creacetee	CARRESTANCE	<b>GRUTATIVATO</b>	<b>PRECIDENCIA</b>	CCTTACK XXXX	CTUCCUCTIFICE	CCGTCCGCAC	ددمحدددده	CCCAACTACA
	TCACGATACT	GASTCCCAGC	GICCCCGITG	CALATAMICC	ACCCGCTTAN	CCANTORCO	GACCCCCCACACG	GACACGCOTO	GGCGGGGGGG	CCCT IN WILL
16001	TTOCANGAAA	RAACTACTTA	GACTCGTACT	CHICFATCHA	<b>PERACRETATOR</b>	CAYSTICKTO!	ACCIONNECTAT	GTCCAMGCGC	MANTCAMG	ANGAGATRICT
İ	AACGITCITT	•	CTGAGCATGA	CAACATACAT	ARKITEGEEGE	CCCCCCCCCT	TOCTTOCATA	CAGETTECCC	TTTTAGTTTC	TICICIACIA

Figure 15J

16101	CCAGGICATC	CCCCCCCACA	ACATACOCC.	CCCTANGANG	CANCINICATE	TAATKETTE	CTCAAAACTA	ANGCODOTCA	ANAMANANA	CTTTCTACT.
				2000	2			Sall		
16201	CANCASTONAL	ACCORDANGE OF THE PARTY OF THE	CATACOARCHIC	CHILL ACTION	CALCULATION	COCACCAGE	CACTOCANAG	GIVEACTACT	AAAACCHITT	TRACER
	TANTA THE		The state of the s	CACATROCTORY			Carl Me crante	CACTURE	TENTROCACAA	Abrya-Truck
16304										
10001	Children		CL.CO. ITARY.	פרורערוני	CACT INCOME		41.000 C		מררופרופ	
	COTOCTOCCA	TCAGANATGC	GOCCACTOS	CGMCTAGGG	CTCKATGTTC	CHURCHCATAC	TACTCCACAT	פככתכופניוכ	CTGGACGAAC	TCGTCCGGT
16401	COARCOCCTC	GRECONCITIO	CCTACCGAAA	CACCICCATANG	CACATARCAREC	CENTRACIACT	GGACGAGTAC	ANCECANCAC	CTAGCCTAM	<b>OCCUSTANCE</b>
	OCTOCOCOLO	CCCCTCAAAC	COATCCCTTT	COCCCTATTC	CTGTACGACC	GCAACOGCGA	CCTGCTCCCG	Troogramme	CATCOCATIT	CCCCATTIGI
	TA A									Kpmt
.000							-	Second Second Second	Constitute & Contract	Ballione Learne
TACAT	CHECHICAE	ACCACOCACO	CCAACOTOGC	NGGCTTCTTT	TCYCOCC(T)A	THICK SCOT	AGACCACTCA	ACCONCOTO	GCACOTCOAL GCACOTCOAL	TACCATRACT
16601	AGCOCCAGCG	ACTOGRAGAT	GICTIGGAM	ANATGACCGT	CONVECTOR	CTERCARACECE	AGGTCCGCGT	GOGGCCAATC	ANGCAGGTUG	CCCCGGGACT
	TCGCGCTCGC	TGACCTICTA	CAGAACCTTT	TTYACTORICA	CCTTCGACCC	_	TCCMACCGCA	COCCOUNTAG	TTCGTCCACC	OCCOCCETGA
16701	CONTRACTOR	STATE STATE OF STATE	THEADATACE	רארדארנייד	AGCACCACTA	THECKACCE	CACAGAGGGC	ATOGRADACAC	AVACGICCCC	GOTTOCCTCA
		-	Abore	CHICANOLTE A	The state of the last		Carrier William	PACE TOTAL	THROCARDOD	PCAACCAACT
	recovered a		31417194	51.515			010111010			
16801	0ccortocco	_	OCAGGCGGTC	OCTUBIORICO	CONCENSAC	-	GITTCAAACOG	ACCCGTGGAT	GTTTCCCGTT	TCAGCCCCCCC
	COCCACCOCC	TACOOCOCCA	COTCCCCCAG	CCACCCCCCC	<b>CCAGGGTTCTG</b>	GAGATICACTIC	CACGITINACC	TOGGCACCTA	CANAGEGENA	ACTCGGGGGG
16901	000000000	CCCTTCCAGG	MAGTACOGCG	CCCCCAGCGC	<b>GCTALTISCCC</b>	DANTATRICCC	TACATCCTTC	CATTOCOCCT	ACCCCCGGCT	ATCGTGGCT
	נכמכמפמכמב	<b>OCCARACTIC</b>	THEATGCCOL	OCCUCACIOCO	CCATCACOC	CTTATACGUG	ATCTAGGAAG	GTAACGCCGA	TCCCCCCAA	TAGCACCGAT
17001	CACCTACCGC	CCCAGAAGAC	DADCANCTAC	CCGACGCCGA	ACCALCACTG	משכבכפנכם	CCCASCASTACGC	CENCECCAGE	CCGTOCTAGE	CCCGATTRCC
	OTOGATOCCO	OCCUPATION	CTCCTTCATO	<b>GOCTISCOCCT</b>	TROTOCTOAC	CTTONOCOCC	COCCCCACACC	GCAGCGGTCB	GOCACGACCO	COCCENANG
17101	CTCCCCAGG	TOCTCCCA	ACATAGGGCAGG	ACCUTOSTIC	TRECONCARC	CHECTACCAC	CCCMATATEG	TTTAAAAOCC	CONCINION	<b>OTTCITICGE</b>
1	CACOCORCIC		recreesee	TRICCACCACC	ACCESTIGACE	CCCCOATCCTO	<b>COCTCCTARC</b>	AAATTTTCGG	CCAGAAACAC	CAAGAACGT
										Springs
17701	ATAMAGENT	CACCOTACCOC	CICCOTTICC	COCTACCOCC		ATTCCCACCA AGAATGCACC	GTAGGAGGGG	CATGGCCGGC	CACGGCCTGA	COCCUARCAT
	TATACCOCCA		GAGGCAAAGG		TANCHOCT	TYTACKTO	CATCCTCCC	GTACCOOCCO	OTGCCGGACT	<b>OCCCCCCTA</b>
	45				Hote					
11104		Charle Course		التقديدي لأردين	S. Vina Care	CATACATA	COCCECT	ATTCCACTOA	TCCCCCCCCCC	CATTEGCCACC
100/1	ייייייייייייייייייייייייייייייייייייייי		Second of the se							CEABOTTSOE
	COCHOCHOOC	orcarocco	ככפכבפנפכם		_			10000		
17401	GTUCCCOGAA	TTGCATCCOT	<b>GCCCTTCCAD</b>	<b>GCCICNGAGAC</b>	ACTICATTANA	AACAAGTTAC	ATCTACINAL	ATCAMATA	MACATCAGA	CTCTCACCA
	CACOCCCC	AACCTACICA	CCCCMCGTC	COCCICTOR	TCACTAATT	TTGTTCAACG	TACACCTITI	TAGITITIATE	TTCAGACCT	GROWITCCTA
										EcraN
17501		ROTANCTATE	TTGTAGAATG	GANGACATCA	ACTIFICACIONE		PLTORICCEG CGACACGGCT	CROCOCCOGITI	CATCOCIARC	TROCMETATA
	OCCUMICADO	ACATTCATAA				AGACCITION	<b>GCTGTGCCGA</b>	OCCCCCCCCAA	GTACCCTTTG	ACCUITCTAT

Figure 15K

1601	ACCCACCAG	CHATATGAGE	CCACCGCCCA	TCAGCTCAGG	CACCIONING CANCING CAN	AGCCCTAAT TITTAAAACC	MANATTANASC	TICCACCION	ANDANCTATO	GCARLANGO" CGTCGTTCT!	
7701	CTOGAACAGC	-	AGATTICTTONS	CCATAANTTO	AAAGAGAAA				CCTCTGGCAT	TAGCOGOOD:	
	GACCTTOTCO	reotoreceo	TCTACGACTC	CCTATTICANC	10401	TMARKTHOF	TTTCCACCAT	CTACCGGAGC	GENGACCGFA	Areaccean.	
7801	OTOGACCTOO	0	ACTGCAAAT	AAGATTAAGA	GTAWA:TTGA	Treecoccet	CCCTTAGAGG	AGCCTCCACC	CCCCOTOGAG	ACAGTGTCT.	
1901	CACCIONALL	TOTALABAM	remain in in	CTCACAGGA			TWINGARE	TCCCTCGTAC	GACCACCAC	TAMAGECANY:	
	GICTCCCCC	_	-	CACHETECET			ATCTOCTOGO	AGGGAGCATG	crecrecto	ATTECTIC	
8001	ccrocccacc	ACCEPTICE	TEGESCECAT	CCCTACTOGA			COTAACCCTC	CACCTCCCTC	CCCCCCCCC	CACTICAGICA	
	CCACGGGTGG	TOGGCAGGT	AGCGCGGGTA	AGCGCGGGTA . CCGATTX:CCT	כאכניאכניכניפ	Techniming	GCATTGCGAC	CTGGACGGAG	GGGGCCGCC7	GINIOCITOCITI:	
1018	AMCCTOTOC	TOCCAGOCCC	CACCOCCOTT	GTTGTAACCC	CHCCTARCCG	COCCITACETO	כפכנפנפנפטנפ	CCAGOSOTCC	COCTAGGAAC	COCCOCATO	
10Ca	TITION OF THE PARTY OF THE PART			CCATCGTGG			ANCOCCOANO	ATECTICIOA	TARCTACOT	Greenwich	
	GOTCACCGTT		_	CCTAGCACCC	_	GITTAGGGGACT	TOCOGOCTINE	TACCANGACT	ATCOATTOCA	CACCATACA !	
8301	TOTCATOTAT		COCCOCCAGA	CHARCTECTO	Acticitate	CCCCCCCCTTT	CCANGATOSC	TACCCCTICO	ATCATOCCCC	ACTOCHETTA TEMENDA: F	
8401	CATGCACATE		ACCCTCGGA	GTACCTGAGE	•	TCCNSTTRC	CCGCGCCACC	GAGACOTACT	TCACCCTCAA	TANCARGETTE	
	GTACGTOTAG	_	TOCOGAGCCT	CATGGACTCG	_	MICHI MANCO	المراشر	CICIOCAIGA	COMPAGNET	ACTION AND A	
8501	AGABACCCCA TCTTTOOOGT	COSTOSCOCO GCCACCOO G	TACCCACGAC	GTGACCACAG	ACCIMENTER	COCAMETICA	GACCCCAAGT	ACCUTOTOCA	CCCACTCCTA	TCACCCATGA	
.0601	COTACAAOOC	COCCAROTOR	CTAGCTOTGG	CACTATACCS	TGTGCTGGAC ACACGACCTG	ATCCCANCIT	CCATGAMCT	CATCCOCOCC	CACCACCTOF	CCCCCCCTA	
8701	AAATTCGG	-	CHECCTACAA	CCCCCTGACT	CCCAAGGGTTG	CCCCANTCC	THECOANTED ANCOCTTACC	CTACTTCGAC	CTACTGCTCT	TGAANTNAN ACTITATITI	
8801	CTACAAGAAG		CAACCAAGAC	CANGINGACC	NACANIAL TISA TUGTTECIAL T	COTCOPPTT	ACTCACCITAT TYACITYCATA	THOOCCAGGC	OCCTFAITCT CCCAATAAGA	<b>GCTATANATA</b> CCATATITAT	
1068	TTACAAAGGA		ATAGGTOTEG	AAGGTCAAAC	ACCTNANTAT	COCCIATITE	CATTICARCE	TOAACCTCAA	ATACCACATA TATCCTCTTA	CTCAGTWGTA	
19001	CCAAACAGAA	ATTACTO		AGTECTAMAA		CANTGAAACC	NT STACGGT TACANTOCCA	TCATATOCAA ACTATACGTT	AACCCACAAA	TCALANTCO: ACTTTTACC"	
1016	CCCUTTCCST	AAGAACATTT	COTTOTTTA	CCTTTCGATC	AAACTECAACT TTTCACTTCA	CHIMANTOCAN	MANAGAR	CTACTGAGGC	AGCCGCAGGC	AATGGTGAT ·	
19201	ACTTICACTICC TCAACTCAGG	TAMAGEGETA S ATTECACCAT	TTGTACAGTO AACATGTCAC	AAGATGTAGA	TATAGAAAGG	הבאמאכאהדכ מיזיכדמדמאס	ATATATACTTA TATAMGAAT	CATGCCCACT	ATTAAAGAAG TAATTCCTTC	CATTGAGTCA:	

CCCATTATAT	TCCATTCATIVE ACCTAACCA	PROAN'S STIT	FCCTACAGAA ACCATGTCTT	MACATAGECO :	NOGCTCCCY*	GGACGCGATO	GGCCCGAGTA	KOTTTCATA: FCAACTATI:	DAMTTOC!	GCGCCGACTT.	PACCETIGATA	CAMCTITAA	FINCUGATOR	CATITATOTI	GOCACOATTO	TCCAGTAAGT
NCANCAGENE GGG TOTTGTEGTG CCC	HITCCTTCAT TCC	-	• -	CCTOTACTCC AND COACATGAGO TTC		-	CCANCACACA CO			CCCCCAAAG GC	• • •	CCCCAACON OF CCCCATCCT CA			CIGICCOGAT GO	CATCCCATTC TO
CTANTCTATT ME	CATACCAGET TO			CCTCTTTANA G			TTANANCCT C AATTITTEGA G	CCANCTOCCT C	· ·			GCCCNCCTA GCCCACCAAT	TTTACGATCG	GOCACHCCAC	ATCCCCCTTC TACCCCCTTC	CCCTTTOCCG
NAATACEA AAAATAA	_		AACAGGTCAG	COSTICOACA	ACACCTACGA	CAACCCATIT	TTCTTTGCCA ANGARACGGT	ATGACCTAAG TACTGGATTC	CATGCTTAGA	TCCATCCCT AGGTAGGGGA	ACACCTACTC TGTGGATGAG	TCCCAATCAC ACCCTTACTO	AAGGACCATO	AGCCCATGAG TCGGGTACTC	POCCOCCACC ACOCOCOCPOS Prof	TVICTATEOTA ACCETAGECT
TENEXEMINA 1						TYTACAACGT ACCTOTTGCA	GOCHCATC	TCCCTAGGNA	CCCTTGAGGC	CCACCCCATA	CTCCCATAT	PCAIRCHUREC NOTICHACCOG	CAMAGACTING	AGANACITICE TCTTTCANCO	TTGGCTACCT	AAACHTRCTT TTTCAAAGAA
TACATHOCTT 1			CTTACCAARS			CTTGACTATA	ACATHICAGET TETANGTOCA	PSII TCTITTAGAGE AGACGTETEG	ACCIGCOTOCA TOXXCXIAGGE	ACTICATACCAA TECCATOOTT	CTCCCCCTAC GACCCCCATT	GACTCTTCTG CTGAGAGAC	CATHGTACHS	CHCCTTCTTT	TCTOSATTES AGACCTAAAC	TTACCCAGAA AATGGGGTUTT
CACCCCTAAT 1			TICCGACAACT			ACCCTOSTCC TOCCACC	CACCOCANCE	TTAACATOGT AATTGTACCA	CCCCCCCACAAC	ATACCCGCCA TATGGGCGGT	CATCACTGGG GTAGTGACCC	CATTACCTTT	<b>GENERACE AND CARCES CONTRACT</b>	ACCOCATOTA TYCCOTACAT	ACACAACAAC TOTOTTGTTO	CAACTCTCCT
CTATRICTICAA C			- · .	AAGAGITTGGA		ACCTTOGACE TOGAACCTEG	TOGTCGCTAT ACCAGCGATA	ACCANGINE	TETTCCCAT AGAAGGGTA	CCACATACCCT CCACATOCCA	ANGRIAANCEC FIECTIFICOS	AGANGOTICOC TETTICCACCO	GOSTINGNAC	ACCTACAAGG TCGATGTTCC	PCCTACACCA AGGATGTOGT	CAAGACCATA
			• •	AMATEMAT		ACCATOTACT	POCTODICAN ACGALCECTT	CHOCAACTIC	TACCCCACET ATCCCCCTCCA	CCCCCACAT	CCTTANGACT	CACACCTITA	TTGACCOGGA	TATCCCAGAG	CAGOTOGOCA	COCTTATAGG
<b>P</b> I	ם ט	CCACANGACC O	y F	TITICAGATA I	. K B		5.5	St	ES	TATCHCTCCO ATAGAGAGACC	CETTEACOCO	TTACCTICANC AATOGNOTTO	MOCOCTCAG	ACCOCTTCTA	CCTCATGGTT	TECCETATE
19301	19401	19501	19601	19701	10861	19901	20001	20101	20201	20301	20401	20501	20601	20701	20801	20901

Figure ISM

1001	TATOTOCAT	GOCCOCACTC	ACAGACCTRIG	GCCAAAACCT	TUTTAL ACTOR	AACTECECTURE 1	ACCCCCTACA	CATCACTUTE (	CHECACETAG	CCATCCACGA	
1101	GCCCACCT	, ,				-			TOTACCTUCO AC/ "XCACOC	CACGCCCTT :	
1201	TOGGCOOCA	ACCCACAAC		ANCCANCATE					AAAGCCATTG	TCAMGATCT	
1301	AGCCGGCCOT		TOTOCACCTA	TYACAAGCGC	TTTCCAGGCT	-	ACACAAGCTC	OCC-TOCOCCA OCCA OCCA OCCA OCCA OCCA OCCA OC	TAGTCAATAC		
1401	GAGACTGGGG CTCTGACCCC	COUNTRACTO	CTACCOSTATE CTACCOSTATE	GCCTFXCIACC COCACCTFGG	CTCACTTANA	AACATGCTAC TTGTACGATG	CTCTTTCAGC		TTCTCACCAG	COACTCAAGC	
10513	AGGITTACCA		GAGTCACTCC	TCCCCCTAG ACCCCCCATC	CCCCATTCCT	TCTTCCCCCG	ACCOCTOTAL TOCCOACATA	AACGCTGGAA TIGCGACCTT	MOTECACEC TTCAGGTGGG	AAAGCGTAC . TTTCGCATGT	
10917	CCCCOONTO	• -	GROCACTATT	CHECTECATE	TTTCTCCACG AAAGAGGTGC	CCTTTACCAA	CHOCKECOA	ACTCCCATGG TGAGGGTACC	ATCACAACCC TAGTGTT000	CACCATGAN' GTGGTACTT	
11701	CTTATTACCG GAATAATGGC	Kprd GOOTACCCAA CCCATGGOTT	CTCCATCCTC	ACAGTCCCC	AGGTACACCC	CACCCTGCGF	COCANCEASO OCCUPIOSTICC	AACABCTCTA	CACCITICATO	OAGCGCCAC" CYCGCGGTA	
1801	COCCCTACTT	CCCCAOCCAC	AGTOCCCACA TCACGCGTCT	TTAGGAGCGC AATCCTCGCG	CACTICETET	TOTCACTTOA ACACTGAACT	AVARCATGTA TTTTGTACAT	AAAATAATOF	ACTACIANACA TOATCIUTOT	CTTTCAATAA GAAAGTTA1*F	
1901	ACCOUNTACT		ACACTCTCGG TGTGAGAGCC	GIGATTATTT	ACCCCCACTC TXXXXXCTCOG	THYCCGRETTS	COCCOFTTAA		CCANGACOCC	COCATCOCTA	
1002	TOCOCCACTO	COTCCCTOTO	OTTOCCATAC CAACCCTATG	TOGTGTTTAG ACCACAAATC	TYPCTCCACTT ACTENACTIGAA Ecnity	AAACTCAGGC TTTGAGTCCG	ACAACCATCC TGTTGGTAGG	COCCOTCCAO	CCACTICAAA	TCACTCCACA AGTGAGGTGT	
12101	CCCACCOCAC	CATEACEAAC	OCCITITACCA CGCANATCOT	CCAGCCCCCC	CCATATCTTG	AAGTCGCAGT TTCAGGGTCA	TOROGCCTCC ACCCCGGAGG	OCCCTOCOCO COCCACOCOC	CCCCACTOC	CTATISTICAL!	
12201	GTTGCAGCAC	• -			CHARCCAGGA	CCCACATACAC	CCTCTAGATCAGA CCTCTAGTCT	TCCOCOTOCA AGGCGCAGGT	GCTCCTCCGC CCAGGAGGCG	GTTGCTCAGG CAACGAGTCC	
12301	GCGAACGGAG		TAGCTGCCTT	CCCANNAAGG	CECTOTOTIC	ACKICTTTCAG TCCGAAACTC	TTGCACTCIC		CATCAAAAOO	TGACCGTCCC ACTCCCACCC	
22401	COCTCTOCOC	CANCETATE	ACCCCTOCA TCCCCOACGT	TAMARGCETT	GATCTGCTTA	AAAGCCACCT	CACCCATACC	GCCTTCASAG CGGAACH: TC	AAGAACATGC	COTAGACTT	
22501	OCCOGNANC COCCTTTTO		Stil GACAGGCCGC CTGTCCGGCG		GREGITICADO CARCASCITIO COTCROTICITA CAGCACOTAS GEOTIFICADO GENECOLOM		CCTCTAGACT	ACCACATITIC TOGICHAAAG	GGCCCACCG	GHCTTCACG	

Figur 15N

	ATCTIGGCCT		_				ATTICABILA	CONCENCT !	ATTITATIONAL	ATGCTTCCGT
TAGAACCGGA		ACGATCTGAC	GACHTANTACTO	כנכסיפעינים	אייראטאטאנאיין א	C. M. P. M. M.				17-1 -
GT B CAC B CT		AAGENCECT	RECARE TEAG	CTS_NGTGGTG	CWXCACAC (	מימיאובכרת י	TOCATION	ATCCTTCTAG	GICACCTOTO	CNANIGALTO
CATCTOTCA		TTCOAGCOGA					ACCEGAGEAC	TACGAACATC	CACTGCACAC	פודיהיכיתיאי
E.	- 1	Pal								
CAGGTACGCC		TOCAGGAATC	OCCICATOAT	CCTCACAAAG	CICTIONING .				CCTCGTTCAG	CCMSSTCT1.
GTCCATGGG	В	ACCITOCITAG	COCKOTAGEA	OCAMBOTTEC	CARANCANCS				GCACCCAAGTC	GILLALMA
CATACOOCCO	8	CCAGAGCTTC	CACTICKTICA			-			CATCAGCGCG	CIRCAGET
GTATOCCOOC	R	GETCTCGAAG	GTGAACCAGT	CCCTCATCM	ACTITIC MAGGG	CHANTETAGE	AATAGGTGCA	CCATGAGCAG	פואנורטרפנ	מתפרוורים ו
				C. B. B. B. B. B.	Cristinal Park	Accelant	CACTITICCOC	TECENOOR	remeeter	CCTCTTCCC
	3	CACCCACCA					CTGAAAGGC	AAGCOACCCO	AGAAGGAGAA	CICAGONICOCO
CONTRACTOR	2	CTREATERACTO	OCHECACINE	ATTCACTOC	recactrate	OCTITACCTICC '	TTTCCCATCC	THENTHAGEA	CCOGREGOTT	<b>OCHEMNACITY</b>
DOCUTATION	150	GCCCCOTCAC	CCAGCAGAAG	TANGTECACE	CCCTGACACG	CONNTCANTO	ANACOGTACO	AACTAATCOT	<b>GGCCACCCAA</b>	CCACTTICAN
ţ	ACCATTIGIA	GCGCCACATC	TICICITICE	TCCTCXCTGT	CCACCATTAC	-	GACGACGCT	COCOCETICOC	ACANGGGCGC	TICTIFICE
Ž	TOGTANACAT	COCCONTING	ARGAGAAAGA	ACCIVITIONCY	GETOCTANTO	GACACCTA	CCCCCCCCCA	<b>ACCCGANCCC</b>	TCTTCCCGCG	ANGALANAGA
2	TCTTOGGCGC	ANTOGCCANA	TCCGCCGCC	ACCTOUNTED	ceseconocte	CONTRACOCO	GCACCAGCGC	GICTIOTOR.	GAGICITICET	CONCCINCOLA
S	AGAACCCGCG	TTACCCOUNT	AGGCGGCGCC	TCCAGCTACC	<b>BOCOCCCCOAC</b>	CCACACCCCC	corocreace	CAGAACACTA	CTCAGANGGA	GCAGATAGCCF
8	CHURATACOC	COCCICATOC	<b>OCTUTION</b>	GOCCOCCCC	COMPROPICE	GCCACCOGGA	CHARGACOAC	ACOTCCTCCA	tocmocoo	ACCTCCCC
¥	CAGCTATGCG	CCCCACTACC	CGANANACC	CCCCCCCCCCCC	CCACCOCCCC	מיכומנכננד	מכככווומכום	TOCAGGAGGT	ACCAACCCCC	TOCAGCGC
8	OCACCOCUTC	COCCUCOCO	CONCOUNTED	COCTOCTCCT	CTTCCCGACT	GECATTICC	TICHEL TATA	GOCAGAAAAA	GATCATGGRG	TCAGTCGAGA
CCTOOCCC	CCAG	OCCCOMOCCC	CCACCANGC	GCCACCAGGA	GANCOCCTCA	CCCCTAAAGG	NGAGGATAT	cconcurry	CTAGTACCTC	MOTCAGETE
5	AGAAGGACAG	CCTARCCGCC	CCCTCTCAGF	TCCCCACCAC	CHASTCCACC	GATOCCOCCA	ACCCCCCTAC	CACCITICCC	Gredabocae	CCCCCCTTGA
Ę	ICTICCTOTC	CONTROCCO	GOGAGACTICA	ACCCCTCCTC	GEIGENGENAG	CTACGGCGGT	TRICECURINT	CTCCAACCCC	באושרובייים	מאשורפישר
	GOAGGAGGAA	_	ACCACCACCC	ACCTITITETA	ACCGMENCO	ACCAGGACCO	CTCAGTACCA	ACAGAGGATA	AAAAGCAAGA	CCAGGGACAA
5	CCTCCTC		restectode	TCCANACAT	TCCCFTCTCC	TOCTOCTOBO	GAGTCATOOF	TOTETECTAT	TITIOGETICE	Concernation in
	•					THE PURE THE PROPERTY OF	CHORGAGACTO	ACGRECATOR	GAAGCATCTG	CAGCGCCAGT
3	OCACAGGCAA		AGTCGGCCC	COCCACCACA	COCATONICAL T	GATGGATCTA	CACCCTCTOC	TECACCACA	CTTCOTAGAC	GPCGCGGTCA
ָ בַּבַ	CONCICCENT	TOCICITION	TOWERCOCK	CCCCTOATCE	STATE OF THE PARTY.	ATAGGGGANTO	TCAGCCTTGC	CTACGAACOC	CACCTATTICE	CACHOCOCOCT
GCGCCATT	CCCCCATTAC	CHOCOACGCO	AACCTICICO	COTCOCTACA	CHANGAGGGS	TATCGCCFAC	AGTECHIANCE	GATGCTTGCG	GTGGATAAGA	OTGGCGCGCA
		_	ACCOUNTS	CCAGCCCAAC	CUCCUCCACA	ACTICTACC	CUTATTRACC	GTOCCAGAGG	TOCTHOCOLAC	CTATCACATI
7555555T	TOCOCCUTT		TOCCUTOTAC	acteograms	מסכסכסמענד	TCAACATCAG	<b>CCATANACOO</b>	CACGOTOTOC	ACGAACGOTO	GATNOTOTA: Ecofiv
									Contraction of the Page	COTTON TATES
HTTEC!	CAR				-		CAGCTGGCCT	ACTIVITY OF	GCGACAGTAT	GCACTATARY
AAAAAGGTTT	E	TGACGITCTA	TOCOCATAGG	ACCICCACCIT	1GGCG1CLAC	אנייים אין	בייייייייייייייייייייייייייייייייייייי			i

Figure 150

## pMRKAdSgag MER6R2

10090	A CALLED BY	COARTICA	AAAATCTTTG	Acceptance of the second	Acceptance	AMericane	CAAACTATT	CCANCAGGAA	AACAGCGAAA	ATGAMETCA
	SCHOOL SHOPE	GATTERIORE	THITAGAAAC	THE PERSON	Trans.			CCTTGTCCTT	TIGHECETIT	TACT PTCAGT
			XProf		,					
		\$	***************************************							
24301	CICIODADIO	TOTOGRAC	TOTACCUTCA	CAACGCCCCCC	CTAMICCITAC	TAMARTACAG	CATCANATIC	RICCALTITO	CCTACCCGGC	ACTTACACCT'A
	GAGACCTICAC	ACCACCTTO	ACCTCCCACT	GTRANKORO	GATYTGCATY	ATTITIONS	GTARCTCCAG	TIXCHOLARC	GCATGGGCCG	TRANTINGENT
24401	CCCCCCAAOO	TCATGAGCAC	AGTCATIGAGT	GARCTICATEC	THICHCOINC	מבאשרניברה	CACACOCIATO	CAAATTTGCA	ACAACAACA	כעננטעטנני.
	OCOCOCITICE	AGTACTEGIO	TCAGTACTCA	CTCCACTARC	ACCENTRACACG	CONTRACTOR	CTCTCCCTAC	GITTAAACGI	terrentror	CHICHCCCIA
24501	TACCCCCAGT	TOCCCACGAG	CAGCTAGGG	CCTONCTICA	AACGCCCCAG	CCTORCOACT	TREGARGAGED	ACCCAAACTA	ATGATGGCCG	CAGTI CTCCT
	ATCCCCTCA	ACCOCTOCTC	GTCGATCGCG	CCACCCAAGT	THEOREGETC	CONCONCITOR	ACCTCCTCGC	TOCOTTTGAL	TACTACCOGC	GTCACGAGGA
		25	app.							•
24601	TACCGTCCAG	-	CTTGAGTGCA TGCAGCGGTT	CTITIOCTUM	CCGGACACATTAC	ACCCCAMACT	ACACTANACA	TTCCACTACA	CCTTTCGACA	<b>GOCCTACGTA</b>
	ATGCCACCTC	CHACTCACGE	ACOTOCCCAA	CHANCGACTG	GGCCTCTACG	TCGCGTTCGA	Terecentrat	AACOTOATOT	CONNECTOR	CCCCATCCAT
		Bru								
24701	COCCAGOCCT	8	CAACGTGCAG	CTCTCCANCC	TRESTUTE	CCPTICICAATT	THECACGANA	ACCOCCTTOO	GCAMACGTO	CTICATTCCA
	GOOGLECOON	COTICTADAG	GTTGCACCTC	CACACOTTOG	ACCAGAGGAT	GCAACCTTAA	<b>MCGIOCITI</b>	TOOCOGAACC	COTTTTGCAC	GAAGTAAGGT
		Asci	ì							
24801	COCTCAAOOG	CONGOCCCCC	COCCACTACO	TCCCCCACTC	CCTTTACTTA	TETETATOLT	ACACCTIGGCA	GACOGCCATO	OCCUPATION	ACCANTACT
	<b>BCGAGTTCCC</b>	OCTCCOCGCG	OCCUTGATOC	AGCCCTTGAC	GCAAATGAAT	AAAGATACGA	TOTOGACCGT	CTOCCOOTIAC	CCCCAAACCG	TEGTEACGAA
			PSII							
24901	GCAGGAGTGC	MCCTCAGG	AGCTCCACAA	ACTCACTARAG	CINAMICTICA	AGNACCTATG	CACCICCTTC	ACCAOCCCT	ccenoccooc	OCACCTORY:
	CCPCCTCACO	TICCAOTICC	<b>PCGACGRETT</b>	TCACCATTTC	GTTTTCAACT	TCCTCSCATAC	CTOCCOOMB	TIGCTCGCGA	<b>GOCACCOOCG</b>	COTOGACCO:
25001	GACATCATT	TCCCCOAACG	CCTGCTTABA	ACCCTUXCAAC	MASSICTORIC	ARACTICACC	ACTICAAAGCA	TOTTGCAGAA	CTTTAGGAAC	THENTOCTIN.
	CTOTAGTANA	_	GGACCIANTTT	TOCCACCITG	TEECHGACGG	TCTGAAGTOG	TCAGTTTCGT	ACAACGICIT	GANATCCTTG	AAATARKATK.
25101	ACCOCITCAGG	ANTOTTOCCC	<b>GCCACCTOCT</b>	GICCACTICC	TACKCACTIT	GTGCCCATTA	ACTACCCCCA	ATGCCCTCCO	CCCCTTTCCC	GCCACACTA
	TCCCGAGTCC	TTAGAACGGG	COSTOCACCA	CACGTGAAGG	ATCCCTGAAA	CACGGGTANT	TCATGGCGCT	TACOCCAGOC	OCCCANACCC	COCHCACCAT
	ged									
25201	CCFFCFGCAD	CTAGCCAACT	ACCETOCCTA	CCACTICTISAC	ATAATCCAAC		TCACCOTCTA	CHOGAGRATIC	ACTIONCOCTO	CACCTATIC
	CONCACCTO	GATCOGTICA	TOGAACOCAT	GGTGAGACTO	TATTACCTTC	TOCACTORCE	ACTOCCAGAT	CACCTCACAG	TGACAGCGAC	GTTGCATACY:
						2	, mark	Pul		
25301	ACCCUBEACE	OCTCC/1007	TICCAATICO	CACCTCCTTA	ACCARAGICA	ANTTATCAST	ACCTITICAGE	TOCAGGGGTCC	CTCOCCTGAC	GANNACTCCTI
	TOCOCCATOO	_	AACGITAAGC	GTCGACGAAT	TOCTITICAGE	TTAATAGCCA	TOGAMCTCG	ACOTCCCAGO	CAGCCCACTC	CTTTTT NOW
25401	CONCINCION	GITGAMCTC	ACTECCADOOC	TOTOGACGTC	COCTACCT	CCCANATITES	TACCTGAGGA	CTACCACCC	CACGAGATTA	<b>GOTTCTACGA</b>
	GCCCAACCCC	Ξ.	TGAGGCCCCG	ACACCTGCAG	CCCIAATGOAA	CHICATTERANC	ATCX:ACTCCT	CATGCTOCOG	GTGCTCTAAT	CCARGATIXT
25501	MONCCONTEC	COCCCOCCTA	ATOCOGAOCT	TACCARCTAR	CHIATTACCC	MATTENCAT	TCTTIXXCAA	TTCCMCCCA	TCAACAART	CCCCCANON
	TCTOSTTAGG	GCCCCCCAT	TACCCCTCGA	ATGCCGCATT	CANTAATING	Precediment	ACANCCOSTT	AACCTICOGT	ACTICITIES	המכנגידוכוו
25601	TTTCTCTAC	GANAGORACO	GOOGSTITIAC	THYCACTICCE	AUTROCOGRICA	CCACCTCAAC	CCAATCCCCC	CACCACCACA	<b>OCCUTATIONS</b>	CANCARCTO.
1	AAACACGATG		CCCCCAAATO	AACCTGGGG	TCACCCCCCT	CCTCGAGTIG	CCPTACKXXCG	GEOGEOGOCOT	CCXXCATAGIC	GICCICOCOI

Figure 15P

					•					
25701	GGGCCCTTGC		CCCACCT.NA	ANGANGCING	MX TRANSIC		ניענימעניניעעני	MATACTOCCA	CARTCAGGCA	באראנאיטינאיט
•	CCCOOCAACO	AAGGGTCCTA	CCONCRAFT	TTCTTCCACC	TEXTAGRACION	CLYSTVAGENCE	כיווייכיזכניזכנ	TTATCACCCT	<b>STCASTCCGT</b>	CITCCITCCA .
						l bowilli				
25801	TOCACCACCA	GOADGAGGAC	ATCATCACAGO	ACTROGRAMA	נינידאהאנייאה	מאחנידיבבה	AGGTCGAAGA	GETGTCAGAC	GAMCACCOT	באכבר אכני
	ACCTGCTCCT	CCTCCTCCTO	TACTACCTTC	TGACCCTCTC	GGATIC TOCTIC	CTTCGAAGGC	TCCARCTICT	CCACAGTCTG	CTTTCTCCCA	CTCCTAGC: A
25901	CGCATTCCCC	TOCCOCCO	CCCACAMATC	GCCAACCCGC	TCCAGGATAGG	CTACAACCTC	כתכדכניזכאם	OCCCCCCCCCC	CACTGCCCGT	TCACCACCC
	OCOTANODO	ADCIGOCCOCO	GOTTETTAG	CCONTRACCEA	ACCITICATACC	CATCITICAC	CCGAGGAGTC	crececece	OTCACCOGCA	ACCCCTCC0
26001	MCCGTAGAT	GOGACACCAC	TOUNNECADO	OCCUGINACT	CCAMACAGEC	<b>GCCTGCCTTA</b>	CCCCAAGAGC	NACANCAGO	CCAAGOCTAC	CIXCHCATGG.
	TTOOCATCTA	CCCTCTCCTC	ACCITIOGICC	CCCCATTCA	GOTTICOTICGS	CRECORCANT	COCCITICACO	TIGHTGROOD	GGTTCCGATG	OCCUMPTACCO;
26101	<b>OCCOOCACAA</b>	GAACGCCATA	OTTOCTTOCT	TOCANOACTO	TOCOCOCAAC	ATCTCCTTCG	CCCGCCGCTT	TCTTCTCTAC	CATCACOXCO	TCXXX TPCT
	COCCOTOTT	CHICCOUTAT	CHACCHACGA	ACCITICAGAC	ACCCCCGTTG	TAGAGGAACIC	GCCCCCCCAA	ACAMGAGATO	GTAGTGCCGC	ACCREMENT.
26201	CCOTAACATC	CTGCATTACT	ACCONCANCE	CTACAGCCCA	TACTRICACER	CCCCCACCCC	CAGCHACAGC	AGCOCCACA	CAGAAGCANA	GREGACEGGA
	OCCATTETAG	GACOTANTGA	TOCCAGTAGA	GATOTCOORT	ATGACGTISC	CACCOTCOCC	Greatistes	TOCCOOTET	grenteorer	CCGCTOGCCT
26301	TAGCANGACT	CTCACAAAOC	CCANGALATE	CACAGCGGCG	<b>GCANCARCAG</b>	CACCIANTIACC	<b>GCTGCGTCTG</b>	GCGCCCAACO	AACCCGTATC	GACCCCCCAAA
	ARCOTICEGA	CACTOTITICO	<b>GGTTCTTTAG</b>	GTGTCGCCCC	COTCOTOGE	CICCICCICG	CGACCCAGAC	COCOCOTTOC	TTOCOCATAG	CTOGGCGCTC
26401	CTTADAAACA	GCATTITICC	CACTOTOTAT	CCTATATTE	AACAGAGAGA	GCCCANGAA	CANGAGCTGA	AAATAAAAA	CACCICIO	CGATCCCTCA
	GAATCTITOT	CCTAMANOG	GTGAGACATA	CCATATAAAG	THENEROGIE	CCCCCCTTCTT	GTTCTCGACT	TITATITI	GYCCAGAGAC	CCTACCCACT
26501	CCCCCAGCTO	CCTOTATCAC	ANAGCGAAG	ATCARCTTCG	GCGCACGCTG	האחאהטכסס	AGOCTECT	CACTALATAC	TOCOCOCTOA	CTCTTAAGGA
	OCOCCINCAC	GCACATAGTO	trincocrite	TAGTCCAAGC	CCCCTRIACTIAL	CTTCTGCGCC	TCCGAGAGAA	<b>OTCATITATO</b>	ACCCCCCACT	CADANTHEC .
26601	CTNOTTICOC	OCCUPACIO	ANATHRAGE	<b>GCGNAMCTA</b>	CGTCATCTCC	AFFCORCCACA	CCCGGCGCCA	OCACCTOTTO	TCACCCCAT	TATIONGCAM
	GATCAAAGCG	COCCANACAG	TITABATICG	CCCTTTTCAT	CCACTAGAGG	reaceasors	OGOCCOCOOP	COTOGRACAAC	AGTEGEOGRA	ATACTCGTTC
26701	GAMATICCCA	COCCCTACAT	<b>OTCGACTTAC</b>	CARCCACAAA	TOCKACTICA	COCTGOAGET	CCCCAACACT	ACTCAACCCG	ANTANACTAC	ATGARCGCGG
	CTTTAAGGGT	OCOCOATOTA	CACCTCAATG	Greenerr	ACCCTICAACO	CCCACCTCGA	COCOTTCTGA	TCACTTGGGC	TIATTICATO	TACTCOCOCC
		Fooffy			មិ	Earli The second				
26801	GACCCCACAT	GATATCCCGG	OTCANCOOM	TACOCOCCCA	CCCMANCCCA	ATTETECTION	MACAGACOCC	TATTACCACC	ACACCTCOTA	ATMACCITIAN
	CTOCOCOTCTA	CTATACCCC	CAGITOCCTI	ATCCCCCCCC	CACTITICOCT	TANGAGGACC	TTCTCCCCC	ATAATGOTGO	TOTOGAGCAT	TATTCCAATT
26901	<b>PCCCCGTAGE</b>	TOCCCCACTG	cccroatera	CCAGGAAAGT	CCCCCLCCCA	CCACTCTOGT	ACTTCC(:NGA	GACGCCCAGO	CCGAACTICA	CANCIDAN
	ACCCCATCA	ACCORDICAR	GGGACCACAT	<b>GOTCCTTTCA</b>	CACCCCACCACT	<b>OTTRACACION</b>	TOMOGRACT	CIGCOGGICC	GOCTTCANGT	CFACTGATTO
27001	TCAGGGGGG	AGCTTGCGGG	COCCUTICOT	CACAROOPER	ממוכהככנה	CCACACATATA	ACTCACCTGA	CANTCAGAGG	<b>GCGAGGTATT</b>	CARCTCAACT
	AGTOCOCOCO	TOUNCOCCC	GCCGNMOCA	ототесско	כבאיבשטכב	COTCCCATAT	TOMOTHODACT	GITAGICICC	CCCTCCATA	GPCCAGTTON:
27101	ACCANOTOCOCT	GAGCTCCTCO	CHICCICICC	GICCOGACCE	GACATTTCAG	ATCGGCGGCG	CCOCCOCIC	TICATTICACO	CCTCGTCAGG	CANTECTANE
	TGCTCAGCCA	CTCGAGGAGC	GAACCAGAGG	CAGGCCTGCC	CTCTAAAGTC	TAGCCACCAC	OCCUBECTORS	AAGTAAGTGC	GCAGCAGTCC	GTTAGGATTC
	III.	1								
27201	TCTGCAGACC	PCOTCCTCTG	AGCCCCCCTC	TOGAGOCATT	GCAARCTCTGC	AATTTATICA	CONCINIONS	CCATCOGNET	ACTITION OF THE	CHICHCOCK
	AUALUS IL	ARE TRUMBER		ACCICCOTOR	Ct. i mmm		tel comer			

· Figure 1562

27301	נכונבבפסכב	ACTATCCOOR	TCAATTFATF	CCTAACTTAG	ACCIONATION					GCAGAGCAAC
	CCACCCCCG	TCATAGGCCT	AGTTANATAN	CRATTGAMAR	TRUKKELLE	ניבונישטבנים ו	כיונהריהאלמכ	TRACTTACA	TICACCTCTC	CONCINCIANI
27401	TOCOCCTGAA	ACACCTOOTC	CACTGTCCCC	CCCACANGING					CCCGAGGATC	ATATOTAGE
	ACCIOCACACTT	TGTGGACCAG	GTGACAGCGG	COOLGENCAC	מאאי לאאנים	CHEAGCCCAC	TCAMARCGAT	GNACTTANC	OGUCTECTAG	TATACCICT
Z7501	CCCGCCGCAC	GOCUTCCOCC - TTACCCCCCA	TACCGCCCA	COGAGACTT	מככככים		GTTTACCCAG		TAGTTONOCO	CICINC MCACCO : A
	GOGCCGCGTG	CCCCAGGCCG	AATGGCCCCCT	CCCTCTCGAA	CCCCCATCGG	ACTAAGCCCT	CARATGGGTC	<b>GCCGGCGGACG</b>	ATCAACTCGC	ccrorcer
						Delli				
27601	ccetoranc	TCACTOTOAT	TTGCAACTGT	CCTAACCCTO	GATTACATCA	AGATCITIGE	TOCCATICACT	<b>GTOCHTAGTA</b>	TAATAAATAC	AGAMATTA: A
			AACCITICACA	GGATTGGGAC	CYMMTGTAGT	TCTAGANCA	ACCCTAGAGA	CACGACTCAT	ATTATTATO	<b>1CTTTAAT</b>
27701	ATATACTOO	CCTCCTATCS	CCATCCTGTA	AACTCCACCG	Trimpleced	CCCAACCAAA	CCANGGCGAA	CCTTACCTOD	TACTITITANC	ATCTCTCO .
	TATATOACCC	_	CCTACCACAT	TTCCCCTCCC	MINNSTRACC	COOFFICURITY	CONCOCT	CCAATCCCACC	ATCANAATTO	THENCHOOTS
27801	CHURCATTEA	CAACAGTTIC	AACCCAGACG	GACTICACTET	ACCIACIACIANC	CHCTCCCAGC	TCARCTACTC	CATCAGAAAA	AACACCACCC	TCCTTACCT1
	GACACTAGAT	-	HOGGICTOC	CTCACTCAGA	TGCTCTCTTG	GAGAGGCTCG	AGTCGATCAG	<b>CTAGRETATI</b>	TTOTOCTOO	ACCIVATOGAC
27901	CCGGGAACOF	ACGAGIGCGT	CACCOCCCC	TEXCACCACAC	CTACCGCCTG	ACCUTANCC	AGACTTTIC	COCACAGACC	TCAATAACTC	TOTTTACCAG
1	GOCCETTOCA	TOCTCACOCA	Gracecece	Acotoctota	<b>GATEGCCGGAC</b>	TCCCATTTOG	TCTGAAAAA	OCCURICTOR	ACTTATTOAG	ACMATGGTC
28001	AACAGGAGGT	GAGCITAGAA	MCCCTTAGG	<b>OTATTACCC</b>	NAVXCCACAG	CTACTGTCATO	<b>GTTTATGAAC</b>	ANTTCAROCA	ACTICTACOOD	CTATTICTAAT
	THETECHECA	CTCGAATCTT	TTGGGAATCC	CATAATCCOG	TTRECEGE	CATCACACCC	CANATACITO	TTRAGTICGT	TOMONTOCCC	CATARCAT
		Xfbg1								
28101	TCAGGTTTCT	CTACAATCGO	COPPOSED	ATTENETIGIC	TRATGRATICE	CTITATICIT	ATACTAACCC	TICHCROCCT	AAGOCTCOCC	מככיומניתי ז
	AGTCCANAGA		CCAACCCCAA	TAAGAGACNG	ACACTANGA	CANATARGAA	TATOATTOCG	AACAGACGGA	TECCCAGCGG	COCACCACAC
28201	TOCACATETO	CATTIATION	CACCTITITION	AACGCTGGGG	TCRCCACCCA	<b>ACATCATTAG</b>	GTACATAATC	CTACOTITAC	TCACCCTTGC	OTC VOLCE VC
	ACCTOTANAC		GTCGAAAAT	-	AGCGGTGGGT	TCTACTAATC	CATCTATTAG	GATCCAAATG	ACTOGGAACG	CACTCCCGT
	Kori									
28101	CETTACCACCC	AAAAOGTOGA	TTTTANCGNO	CCACCCTGTA	ATCTTACATT	COCAGCTGAA	<b>CCTANTITAOT</b>	GCACCACTCT	TATAMATOC	ACCACAGN .
	CCATGGTGGG		AMANTICCTC	<b>GOTCGGACAT</b>	TACAATGTAA	GCCTCGACTT	CCATTACTCA	CCTCGTGAGA	ATAITITIACO	POOTGTCT103
28401	ATTOAAAAGCT	OCTIVATION	CACAAAAACA	MATTOCCAA	GEARGETETT	TATESTATES	תוכאסככאסם	TCACACTACA	CACTATAATO	TTACAGITT! F
	TACTITICAR		GIGHTHOP	TTWCGTT	CATACCACAA	NTACCATANA	ccorcoorce	ACTOTOATOT	CTCATATTAC	AATCTCAAAA
			128	Bullon						
28501	CCAGGGTAAA	ACTICATABLE	CHITTATOTA	TACTITICCA	TITITATGAM	TOTRICGACAT	TACCATGTAC	ATCAGCAMC	AGTATAAGTT	GROSCECCCA
	CONCCENTITY			ATCANANGOT	ANNIACTIT	ACACCCTGTA	ATCCTACATO	TACTECHTIO	TCATATTCAR	CACCGROOM
28601	CARANTHER	_		TOCTOCACTO	CTATGCTAAT	TACAGTGCTC	OCTITIONET	GTACCCTACT	CTATATTAMA	TACANARGCA.
•	GETTENCIC	-	ACCOTCAMAG		CATACGATTA	ATCTCACGAG	CCANACCAGA	CATGOGATGA	CATATATT	ATCTTTTCC "
78701	CACTEMOTIC		AACAAAAACC	CTTAATTTAC	TANCTTACAA	ACCTANTATE	ACCACTAACT	<b>OCHETRCICO</b>	CTCCTTCCAA	AACAAATTI
	CTOCCTOCAA	_	•	CANTIMATO	ATTCARTOTT	<b>TCGATTACAG</b>	TACHGATTCA	CGANATONOC	DACCANCOTT	TICITIAN :T
10000	AAAAAAAAAA	_	GAATAGGATT	TANACCECTE	CCHCATTRC	TRETTANTAC	CATACCCCTO	MCANTITOAC	<b>ECTATIONOS</b>	ATATISCICI'A
***************************************	THICARICO		CTIATECTAA		CCAGTAAAGG	ACGAGITTATG	CTAACCCCAAC	TIGITIMETO	AGATACACCC	TATACCACIT

# PHRKAdSgag MERGBZ

. 28901	CCCCTACAAC	CTTGAAGTCA	CCTANGGATE	ATCTCAGEAT	CTGACTTTGAG	CCAGCACCTG CONTINUES	TCCCCCCCCACAT AGGCCCCCTA	ANCANGERCA	CCAACTACAG	CCACCCACTC CCT 125T 111
29001	TAACAGAGAT	GACCAACACA	ACCAACGOGG TOOTTOCGCC	מבפכבמידאר מבכדבבמאדו:	CCCACTTACA	ACATINGCACAA ACATINATA	ATACACCCCA TATGTTCOOT	ACTITICITACE	TTTOTCASTA AACAGTTAT	ACTOOGRATAA TOACCCTATT
29101	CTTOCOCATO	TOGROGITET ACCACCAAGA	CCATAIX GCT GCTATCGCCA	TATISTA	TCCCANTATA	TTATCHEST	CATCTRCTGC	CTANARCCCA	AACOCGCCCC	ACCACCCATC TOGIV ILY: FAG
29201	TATROTCCCA	TCATTOTGCT AGT/ACACGA	ACACCCAAAC		PCTATAGATT AGGTATCTAA	GENERALIO CETGESTERE	ANACACATGE FITCECENCA	ACAMANGAGA ACAMANGAGA	TACAGTATGA	TTANATGAGA AATITTACTCT
29301	CATGATTCCT.  GTRCTAAGGA	CCAOTITITA CCCAOTITITA GCTCAAAAT	TATTACTORC ATAATGACTO	CCTTGTTGCG	CTTTTTTTTG CONSCICOL GAANANCAC GCACGARTIG Pall		ATTOOCTOCO	OPPICICACA CAAAGAGTOT	TCGAAGTAGA AGCTTCATCT	CTCCATTC A
29401	acctrcacao cadaaotorc	TCTATTIGCT AGATAAACGA	TTACGGATTT	CACTOCOLACT	COCTCATCTO CACCOCOATC	CAGCCTCATC	ACTOTOCACA TRACACCAGT	ACCCTTTAT	CCAMBCATT	GACTOGGT.T CTGACCCATA
29501	CACACGCGAA	TOCATATOTO ACCTATAGAG	AGACACCATC TCTGTGGTAG	CCCAGTACAG	GGACAGGACT	ATACCTGAGC TATEGACTEG	TTCTTAGATT ANGMATCTTA	TCTTTANTTA	TOWATTTAC ACTITAAATO	TOTOACTTY
29601	CTCCTCATTA	TTTOCACCCT AAACOTOOOA	ATCTGCGTTT TMGACGCANA	TOTTCCCCGA	CCTCCAAGCC	TCAAAGACAT AGTTTCTGTA	ATATCATGCA TATAGTACGT Pall	CTANGTCHCC	tataticcaat Atatacceta	ATTCCAAGI T TAAGGTTCAA
29701	CCTACAATGA	AAAAAGCGAT	CTTTCCGAAG	CCROSTIATA	TCCAATCATC ACCTTAGTAG	TCTCTTATOD AGACAATACC	TGTTCTGCAG ACAAGACGTC	TACCATCITA	OCCCTAGCTA COCATCGAT	TATATCCC . A ATATAGGGAT
29801	CCTTOACATT	OCCUACCTIVE CCUACCTIVE	CANTAGATCE GITALCTACG	CATGAACCAC GTACTTGGTG	CCAACTITCC	מסכפכפפבם	TATGCTTCCA	CTCCAACAAG	AACAACGGCC AACAACGGCC Xb Xb Bgfff	CUBCTTROTT. GCCGANATA 1
29901	CCAGCCANTC	AGCTCGCCC	ACCTICTCCC TGGNAGAGGG	ACCCCCACTO TGGGGGTGAC	AAATCAGCTA	CTTTAATCTA	ACAMMANGAO, ATGACTOACA TOTOCTOCTO TACTOACTOT	ATGACTGACA	CCCTACATCT	AGAAATKGAC PCTTTACCTG
10001	CCTTAATAAT	CNAAACAGCO	OCTOCTAGAA GGACGATCTT	ACACCCACCC TCTGCGTCCC	CAGCGGCCGA	CENTERCEC	ATGAATCAAG TACTTAGTTC	AGCTCCANGA TCCAGGTTCT	CATOUTTAC	TIGCACCAGE
30101	OCAMAGGG CONTINCCC	PATCTFTTGT ATAGAMACA	_		CACCTACGAE	ACTIVATACCA	CCGCACACCG	CCTTRACTAC	AAGTTGCCAA TTCAACGGTT	CCAAGCOT". GGTTT.TF.ALT
30201	CITTAACCAC	CASTACCACC	CTCTTTTCCC	CAFFACCATA GTAATISCTAT Bytt	ACTCAGCACT TGAGTCCTGA	CCCATCTITIG	CCANCOCTOC GCTTCCGACG	TAGTGAGTG	DAACAGTICC	TOCACTCCTA
30301	CTCTOCACCC	TTATTNAGAC	CCTGTGCGGT	CAGTTTCTAS	TTATTCCCTT AATAAGGSIAA	TAACTMATAA ATTGATTATT	MANANATAA	TAMAGCATCA ATTTCGTAGT	CTTACTTAAA	ATCAGTTAGG TAGTCAATCG

### pMRKAd5qag HER682

30401	MATTICTUT	CCAGTITATI	CAGCAGCACC	ACCENIA COCCE	CETTCEACET	CHCATTAINGC	AGCTTCCTCC	TOCCTOCAA	CHITCTCCAC	ANTCTABATO
30501	CHACACTCA	TECTECTOF	TCCTGTCCAT	CCCCACCCAC	TATCTFCATC	AACAACTECT	TGAAGCGCGC	AACACCCTCT	GARCATACCT	TCAACCCCC:T
30601	GTATCCATAT		CCGOTCCTCC		THETTACTE	CTCCCTTTGT	ATCCCCCAST TAGGGGGTTA	OCCTITICAS CCCAAAGITC	AGAOTICCCC TCTCAGGGG	TOGGGTAC1 :
30701	TCTFFGCGCC	TATCCCAACC	TCTASTTACC	TCCAATGCCA	TGCTTGCGCT	CAAAATRGGC	Menaneter	CTCTOGACGA	GOCCOCCAAC	CPTACCTCC!
	AGAAACGCGG	-	ACATCAATGG	ACCITACE	ACCIANCOCKA	GTTTTACCCG	TYCCCAGAGA	GAGACCYGCT	ccooccomo	DANTRICARY !
30801	AAAATOTAAC TTTTACATTO	CACTOTORGE	CCACCTCTCA	AAAAAACCAA	GAGTETGTAT	AACCTOGAAA	TATCTYRIACC ATAGACGTG3	CCTCACAGTT	ACCTCAGANG TOGAGICTIC	CCCTANCTAIT
30901	CCGACGGCGG	CCTCCTCTAA	TGGTCGCGGG	CAACACACTC	ACCATCCAAT TOOTACGTTA	CACAROCOCO	CCATTCCCAC	CACGACTCCA	AACTTAGGAT	TOCCACCCAA ACOGTOGGT I'
31001	OCACCCTCA	GICACAGICT	AGGAAAGCTA TCC777CGA7	GCCCTGCAAA	CATCAGGCCC	CCTCACCACC	ACCCATACCA TOGCTATCOT	GTACCCTTAC	TATCACTOCC	TCACCCCCT** AGTGGGGGAA
31101	TANCTACTOC	CACTUSTAGE	THOOSCATTO	ACTTORANGA	COCCATTTAT	ACACANANTO TOTOTITITAC	GAMACTAGO	ACTAAAOTAC	CCCCAAGGAA	TOCATOTAL .
31201	ACACCACCETA TCTGCTGGAT	AACACITIGA FTOTCAAACT	CCGTAGCAAC	TOOTCCAGO! ACCAGOTCCA	CACTGATANT	ATANTACTIC TATTATGAGG	CTTGCAACT	AMSTINCTO	GAGCCTTGGG CTCGGAACCC	TITICATICA MANCTAN
31301	CAROCCAATA	ACOTTOAATT	16TAGCAGGA ACATCGTCCT	OCHOATICCE CCTGATICCE	THUATTCTCA	NACAGACOC	CTTATACTTO	ATOTTACTTA	ACCONTIGAT ACCCAACTA	CCACITITAL
31401	ALCTANATOR	• •	CAGGGCCCTC	TTTTATANA	CTCAGCCCAC GAGTCGGGTG	AACTTOGATA TTGAACCTAT	TTAACTACAA	CANAGOCCTT	TACTTOTTTA	CACCTICAA \
31501	CAATTCCAAA	AAGCTTGAGG TTCGAACTCC	TTAACCTAAG AATTOGATTC	CACTGCCANG	<b>GRATICATES</b> CCCAACTACA	TTGACGCTAC AACTGCGATG	AGCCATAGCC TCGGTATCGG	ATTAATGCAG TAATTACGTC	CACAACCCAA	TOAATTTOT: P ACTTAAACCA
31601	TCACCTANTO ADTOGATTAC	CACCAAACAC	AAATCCCCTC TTTAGGGGAG	ANACOMAN	TTYRECATED AACCESTACE	CCTAGAATTF	GATTCAAACA	AGGCTATOGF TCCGATACCA	TCCTAAACTA AGGATTTGAT	COTTOACCIN
31701	<b>TTAGTITTGA</b> ANTERANACT	CACCACAGOT	OCCATTACAG COCTAATOTC	TAGGAAACAA	NATAATOAT TTTATTACTÄ	MACTAACTE	Transported Acacettamo	ACCAGCTCCA	TCTCCTAACT AGAGGATTGA	GTAGACTAAA CATCTGATTT
31801	TUCKGAGAAA	CTACGATITO	TCACTTTGGT AGTGAAACCA	CHTACAAA	TOTAXCHOTC ACACCOTCAG	AAATACTTGC TTTATGAACG	TACAGITICA	CAAACCGAC	TTAMOOCHO ANTTECESTE	TTTGGCTCCA
31901	ATATCHCOM	AFATCHGGAA CAGITCAAAG FATAGACCTF GICAAGTITC	TUCTCATCT! ACCAGINGAA	ATTATAAGAT TAATATHETA	THENCEANA	TXX:AGTGCTA ACCTCACGAT	CTANACANTE	CCTTCCTGGA	CCCAGAATAT	T. AACTITA
32001	GAAATGGAGA	Byn A TCTTACTRAA T AGASTGACTT	OOCACAACCT CCGTGTCAGA	ATACAMACOC	TGTTOGATTT	ATCCCTANC TACTGATTGG	TATCACCITA	ACCITITING	CACCOTAMAA	CTCCCAAAAG

Figure 15T

32101	TAACATTGTC	AGTCAAGTTT	ACTTANACES TRAATTTRAFE	ACACAMACT TO TOTAL TICA	AAACKTGTAA	CACTAACCAT	TACACTAGAC	CCATCTOTCC	AMCAGGAGA	CACAACTC::A GTGTTRAG: 3"
32201	AGTGCATACT TCACGTATGA	CTATGECATT	TTCATCCCTG	TEXT TOTAL AND		TATTCATATA		CCTCTTACAC	THITTCATAC	ATTGCCCAN:
32301	AATAAAGAAT	CONTROLOTA	ATOTTTC/AC TAC/AAGITTG	CACAMTAN	TTCANTITEA	CHITTANACT	NATCATTITE TCAGTANNA	CATTCAGTAG	TATAGCCCCA	CCACCACATA
32401	CCTTATACAG	MICACCGIAC	CTTAATCAA	CTCACACAAC	CCTAGRATIC	ANCETVACAC	CHCCCTCCCA	ACACACAGAG TGTGTGTCTC	TACACAGTCC	PPTF: PCCC1 V:
32501	GCTOGCCTTA	ANAGCATCA	TATCATOCT	AACAGACATA	TECHTAGATA		CACCOTTICE	TOTOGRECEA	AACOCTCATC	AGTOATATI .
32601	ATAMCTECE TATTICAGES	COCCACTC	ACTTARGTTC TOANTYCARG	ATCTCCCTGT	CT.N. R. TON, TG CATTLY CANCGAC				CTTAACOGGC	GOLTHANDIA:
32701	ANGTECACO	CTACATGGG	GTAGAGTCAT	AATCGTGCAT	CNGGATAGGG	CHTINGEST GCAGCAGGG	CHATROTOCT CCACCACCC GCGAATAAAC	GCUANTANAC	TOCTOCCOCC	OCCACHCCGT
32801	Pro			CTCAGCGATG		CCCCRIMENT		פוכבוננסס באכאפכאפס	CACAGCAGCG	CACCCTOA!
32901	PCACTTABAT	CACCACACTA			TATTESTON	AATCCCACAG			GCTCATGGCG	OCCACCACAG
33001	AGTGAATTTA	GECATCATAC	TGACGTCGTG	TCCTGCTGTT	ATAACAAGTT	TTAGGGTGTC	ACCITICATE CREATE	ACATAGGETTE	CONSTACCOC	CCCTCONGIC TGTTGTAATT
	TTGGGTGCAC	COSTACTATO			CACCGCTOOG	GACTATTAGE	GCGACCTGTA	THOTAATOG	AGAAAACCOT	ACAACATTAA Figi
33101	CACCACCACC GTGGTGGAGG	OCCATOOTAT		TAATTTGTAC	CGCGGTACGT	COMPOTACCA			COOCCOCCO	ATATOTCAC:
33201	ACCCT/GOCC	CTOACCTTOT	AFGACAGIUD TACTUTCACC	AGAGECEAGG	ACTICITA ACC TGACCATTOC	ATGGATCATC TACCTAGTAG	MACAGEMAT	ACTATAGITA O	CAACCGTGTT	CACAGOCACA GRUTT:CGTGT PSI
33301	COTOCATACA	CTTCCTCAGG	ATTACARGET TAATGETCGA	CCTCCCCCTT	TACAACCATA	TCCCAGGAAA	CACCCATIC	CHOANTCACE	<b>GTAMATCCCA</b> CATTTAGGGT	CACTICLACIA
33401	MENCETOCC	ACCETANCTICA TOCATTOAGT	CGTTGTGCAT	TOTE AND THE AC	TTACATTCOS ANTOTANOCO	CHARTARITY COLOR	ATGATECTEC TACTACAGE	AGENTOOFNO TCATACCATC	CCCCCCAAAG	TCTCTCAAA ACAGAGTTT F
33501	OCACOTACAC CCTCCATCTO	GATCCCTACT	GTACCOLAGING CATAXCTCAC	CCCCCCCCCCC	ACCCAGATOR TOCCTCTAGE	TOTAL CONTOUR ACMACEMENT	AGTOTCATIC TCACAGING	GTTTACCTTO	OCCOCACOTA CGGCCTGCAT	GTCATATTP: CAGTATAAV

TTATCGROOM **GCTACKXCKAT** CCACACTOAT ACCAPITITION TOCTANAMAG AACAACCCTT ATCTCCCONG TACABACCTC CENTRACTAG CGTCCCXATC AATAGCACCC GTOACCTOTO CCATCACTTCC GCAGTGAAGG GTOCK:1CG.FF AMACCITI MAN TOGATOTOTA RACIAGACOC TCAGTOTOTO CCCTCCTCC CCTTCTCARC CTTCTTGGTA CAMAAAAAA AATAAGGTTT TCTAATAGGT TTTGGAGTTT ACCATCACTO TOCTACOCCT CCTCCACCTT OCAGOTOGAA CONTECEDTA CACTOCATIVE CACAMITOC GGTGTCACT/ GTTTTTACE CATCATATAG CTGAGAGARTI CTOTTIACCC CAMMANTC CACCCAGCC THICHCHANCT CTACACCCAA AGAACAGATA ATGGCATTTO TAAGATGTTO TETTOTETAT TACCGTAME ATTETACME RECTANANCE THEAGRAPHS ATCHECTETA TANACATTEE AGELECTICA CCAGGAMAC GTCCCGGTCG ACTICIANTA GCACITICLAG ACGIGCCTGG TCGCGCCGGT GAAGGGGCGG TCCTTGGTAC TGTTTTCTTG COCTACATTC GAACAACCTA CCCCCCCTA TATTTTACOT TCCACGACGA CONCTACETC TATTICCETIC CATTICOAGGC CTTGGTGGTG TCTTTTTCTG TTGTAATCTT COCACAGAAT GTTGTCCTTT GACCACCCAG CTGGCTTTAT CGGCCCCCT TATGTATGGG mmmon CCANANACE CAEMCTICE TEMATIGIC ACTICCOTT TECCAGGITA CTITICITIE GOTTITIO GIGITOANCO ACTITARCAG TOMOGEAAA AGGIGCIAT Techchoooc GACATTAC ANANTACCO CENCRATED EFFERANCE GCCIGICITA CACAGGAAA CCCTARGCAA COGATCCUTT MANANCAC TAGAGGADAT ATTTOTAGG TCGTGGAAGT AGENCICOCO ACCUANCENTS ACANAGRAS ATAMATOCA AGGIOCTOCT AGANANAGAC CHICACOGNIC OCCCODOGG ATACATACCC **STTAMOTICS** CAATTICAGG GANTAAGCCA CETATICOGE TTATTCCAAA AGATTATCCA COCCOMMAN AGRANGEACH TEOTHOTECHT CETECHTICAG AFANAGEACH GTANGETECG GAACCACCAG ATAGGAGAGA ANACACATA ACACCTIGAA ANACCCTICCT GTCACCOTGA TTANAMACCA CCACOCACAG CONCOCTOR THYOGOACCA ACTIVATIONS ANACCTATT TCACCTAACO ACTOCATION AMMATCTOC TITIODATA THITTAGACG TCTACTACTT ACATCATCAA CACCACCCCA **GROOTGGCGT** ANATAACANA ANANCATTIN ANCATTAGAA CTCAPTETT GACCGANATA THINGTON THOTOGACTE CTATATATAG GACTAAAAA FANGACACIC ATACTICAGAG CTATICCTAAS CACCOTAGIC CEGATOTAAG CTTGTTGCAT GGGCGGGAT preventing whence the date of the detection CTANCCIAGAC TGATAACATC CHARTETER CCCCCATTGT **OCCUPATABLA** CCTCACAGAC CTGTATAAGA TTCAAAAGCO ORCOCTTAOR ACTANCOTTS STANGFICCAA GGAGTGTCTG GACATATTCT AAGTTTTCGC כבוככככככ ANTITUCOT TCATTATATE ACTATTOTAG CCATANCHOT CHONCITAIN אנכראלאנה מניגנאנינים HECTROOTE ESCESSER COARGECEAN GATACATTIC AGGAAGTACG COGGOAGGA TGARCATAAT CGTRCAGOTC TGCACGGACC AGCGCGGCCA TITATION TIMOTAAT CAGTGACACT **GCTANNAGC** DOTATTOTCA OTCODANTOO TICCCOGITC ACGICICACT CATATATAT **APTOCOCANT** GGANGAGCTG GAAGAACCAT ACCAGITICA GAIGICGGIT CCGATTITOGO ANGTECCCACT CANATCCCCA ATATTAAGTC GTTTAGGGCT TATAATTCAG CGATTTTCG Handill MANAMACTO TOCAGAGOGA ころいろんりいころ TITITION ATCOUNTCAGE TACCCAGTCA TATCCTCTCT TTCTGCATAA ACACAAAATA GOCCATOCCO GCGTGACCGT GITGATICAC PCTGAGCCAT TTGTGTAGTC CAACTAAGTG ACCAMATTA ATCCTCCATA TIGITITIMI ECCADAMAC DOCACOCOM ECTACOCCOA GANGGAMA ACCATCAGTA TGTGTTTAT TOTOCOCCOTC ANDORCCANG GATACGATTIC GTCGCATCGG ACAGCGGCAG TATCTCTAAG GOCOTRACAN ACARATCTER DACINCOTITY TOGICCACOC CCCCACTORY TOTICTAGACG CTATGTANG ACTCACACAC GGGARAMGCG **TCCGGTGGCG** GECCONGER ACCECANCE CACCTCCANG TOGACGTAAA OTGCAGGITC ACCTGCATTT GAAGACITAT ATAGAGATIC AATTCAGGTT CTOCCTUATO CCOCTACOCC COCACTOCCA CAGACOCCCA AAGACGTATT remiconer **AACACATCAG** TACCAGGTAT GUNCANCATA CAOCOCTTCC CHUTTOTAL GICGCBANGG CHATCHOTCA CAGTGTANA GTCACATTIT OCOTOCOCTE COATOCOCOT COCOCINCEC CTTCTCMTA TRATTRECARA CTICARIT **OFFINATICAGE** ATGAAGATCT ATTAACTGAA TACTICTAGA TAATITCACTT TTIOCCCOOR TTATTANGAG TAGAGCOGTO CACCCAATCA TATOROCCTC ACAGCCCCCA ACCAGGIGGG ACCTACACAT TOUTICIOCO AAACCGCCCT ATCTCOCCAC COTECETTES CARRECTARCE OCOCOTTITI OPETOCOCOF GACCONCTAC AGACTCOOTA TOTOGOOOF **ACCECCECTO** COCACCADET **DOCULTURA** CACCTCANO OCCAMBOCCE AGAGITITOTA TATICOTATE TCATAATOTA CTCMOCANA AAGCATCCAG PCCAAAAGGC AGOTTICCO ANTANTICIC OFCOGRAFIC ATACTOTOCO CCGTTTCGGA TCTCAAACAT ATAMOCATAA **AGTATTACAT** AGACAACATT **ICTUTIOTAL TCCCOCTCCA** LOCOCCAPOCT CCOTOCTCCA 35001 35101 35201 33601 33901 34001 34201 34301 34401 34501 34601 34701 34801 34901 33801 34101 33701

Figure 15V

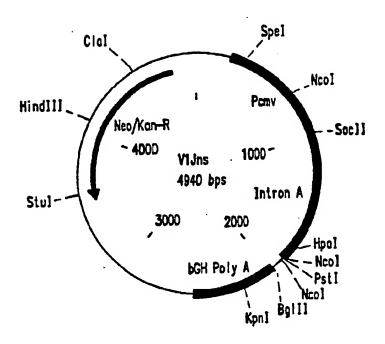
35301	CATTITARAA GTAAAATICT	AAACTACAAT TTTGATGTTA	TCCCAACACA	tacangttac atgeticaatg	NYCKECTAA MKKCFFFFFFF	AACCTACGTE ACCGGCCCG TTGGATGCAG TGAGCGGGGC TTGGATGCAG TGAGCGGGGCCCCGGGGGGCCCCGGGGGGGGGG		TTCCCACGC AAGGGGGGGGGGGGGGGGGGGGGGGGGGGG	CCGCGCCACG	TCACAMETE ACTICITITICAS
35401	CACCCCCTCA	TTARCATATT AAFAGENTAA	CCCANGITAG	CANANTANGG	TATATTATTO	ATTENTEDAY	TTANGANTTC AATTCTTANG	OGATCTOCOA CCTMCACOCT	COCCAGOCTO	GATGGCCTT.
35501	CCCATTATOA	THETHEREGE	TTCCGGGGC	ATCCCCATIC	CCGCGTTGCA	CCCATOCTO	TECHOGENOS AGGTECOTEC	TAGATTIACGA	CCATCAGGGA	CACCTTCAAN
35601	CONCORPER	GOCCADONAC	CCTAAAAAGG	CCCCOTTCCT	GGCGFTFTTC	CATACACTAC	GCCCCCCTGA	CCACCATCAC	ANAAATCCAC	CCACTTCAGE
35701	CACOTOCCAA	AACCCGACAG	GACTATAAAG	ATACCAGGCO TATAGGTCCGC	THECECETS	CHICANGOCA	CCHICCOCTCT	CCTUTTCCGA	CCCTGCCGCT	TACCCOATAC ATGCCCTATE:
35801	CHURCOCCE	TTCTCCCTTC	CCCTTCGCAC	GCGCTTTCTC	ATMRCTCACG TATCGMGTCC	CTCTARGTAT	CHEANTICES	TOTAGGTCOT	PEGETECAAG AGEGAGGITE	CTCCCCACN!
35901	TOCACOMACC	CCCCOTTCAD	CCCGACCGCT	COCOCANTAG	CCCATTCATA	CONCINCAGE	CCAACCCGGF	AAGACACGAC	TTATCGCCAC AATAGCGGTG	TOOCAGCAG".
36001	CACTGOTAAC	AGGATTAGGA	CICGCICCAT	TOTAGGGGGT ACATCCGCCA	CCATCACACAT	TCTTCAAGTO ACAACTTCAC	CACCOGATTO	TACOGCTACA	CTAGANGGAC	AGTATTTGOT TCATAAACCA
36101	ATCTOCGCTC		Agtracctre	CCATATACAC	THAT THAT THE MACE AND MACE CANTERNAL THAT THE MACE CANTERNAL THAT THE CANTERNAL THAT THE CANTERNAL THAT THE CANTERNAL THAT THAT THE CANTERNAL THE CANTERNAL THAT THE CANTERNAL THE CANTERNAL THAT THE CANTERNAL THE CANTERNAL THE CANTERNAL THE	THGATCCGGC AACTAGGCCG	ANCAACCA	CCCCTGGTAG	CCCACCAMA OCCACCAMA	TTTGTTTGC. AAACAAACGT
36201	ACCACCACAT ACCACCACATA	TACGCGCAGA	AAAAAAGGAT	CTCANGANGA	TCCTTTGATC ACGANACTAG	TTTTCTACGO AAAAGATGCC	CCAGACTICO	TCAGTOGAAC	CTTTGAGTO	GTTANGGGA" CAATTCCCTA
36301	AMCCAGTAC	ACATTATCAA	AMGGATCTI	CACCTAGATO	CTITIANAIC	ANTCTANNST	ATATATGA07 TATATACTCA	AAACTTGGTC	TCACAGTTAC ACTOTCAATO	CNATCCTTAN
36401	TCAOTOROOC	ACCTATCTCA	OCCUPACION COCTAGACAG	TATTTCGTTC	TACCATAGET TACCETATCAA	<b>GCCTOACTCC</b> CTGACTGAGO	CCCTCCTCTA	CTATTCATCC	ATACGGGAGG TATGCCCTCC	CCANTRACCAIN
36501	TOOCCCANT	CONCOTTACT	PACCCCCAGA ATGCCCTCT	CCCACOCTCA	CCGCCTCCAG	AFFTAFCAGE TAAATAGTEE	ANTIMACCAD TTATTTGGTC	CCMCCCCCCAA	CCCCORCICO	CACANGROOT
36601	CCTCCACTT	FATCCOCCTC ATAGGCGGAG	CATCCAGTCT	ATTAATTCTT TAATTAACAA	GCCGGGANGC CGGCCCTTCG	THANTANGE	AGTTCGCCAG TCAAGCGGTC	TTAATAGTT? AATTATCAAA	GCOCAACGTT CCCOTTGCAA	CANCOGTANG
36701	CTACAGGEAT	CONCONONICA	COCTCOTCOT OCCAOCACTA Presi	TTGGTATGGC AACCATACCG	TTCATTCAGC AAGTAAGTCG	TCCGGTFCCC AGGCCAAGGG	AACCANTCAAO TTGCTAGTTC	GCGAGTTACA	TGATCCCCCA ACTAGGGGGGT	ACAACACGTT
36801	AAAACCOTT TTTCCCCAA	ACTCCTTCO TCCAGGAGG	GTCCTCCGAT CAGGAGGCTA	CONTRACTOR	ACTANGTING	CCGCAGTGTT	ATCACTCATO TAGTGAGTAC		CACTGCATAA	TTCTCTTACT ANGAGANTGA
36901	GTCATGCCAT CAGTACGGTA	CCCTAAGATG	CTTTTCTOTO	ACTRICTICALTY TRACCACTICA	ACTY ANCOAN TOAGTTOGTT	GAGTAAGACT	CTTATCACAT	ACCCCCCTGC	GAGTTGCTCT	TACCCASCGT

figure 15W

### PMRRAdSgag MER682

CT TACKECTOTT	CC TICCGITTI'A	AT TOTOTOATICA	GCGGATACAT ATTICAATOT ATTAGAAAA ATAAACAAAT AGGAATINIG GCACATATC CCCGAAAAAT GCACCTAGAA CCATTATAAA CCATTATAAA CCATTATAAA CGATAAAAAA CGATAAAAAAA CGATAAAAAAAAAA		00
TCAAGGAT	CAANAACA	TCAGESTET AGTCCCAA	CAGATICE		ID NO: 27 ID NO: 28)
CHITTCAG	TCCCTCACTC	AGCATTTA TCCTAAAT	CACCTGAC		MT (SEQ TA (SEQ
GANGANGICC CO	CACCAGGGTT TO	CANTATTATT GA	CCCGANAME OC GGCTTTTCA CC	Ecriti	THA ACCTATAMA ATRACCIAT CACGARGECE TTECHETE ANGATHEGA TEGRATET TAAT (SEQID NO: 27) AAT TEJATATITIT TATECCEATA GIGETECKES AAAGEARAG TECHAMET AGGETAMB ATTA (SEQID NO: 28)
ATTRIBANAC TAACCITITIG	CTTTTACTTT	CAACTAAAAA	היאראלדאר סכפופדאאאפ	Gamfü	ANCANTIEGA THCTFANCCT
AGTEACTECATE: TEACGAGTAG	AGAMETER AGAMETER	TACTCATACT ATGAGTATCA	AGGGATTTYTE TCCCCAAGAC		TTICGICTIC AAAGCAGAAG
GAACTTTAAA	ACTEANETES TEXASTECACT	AAATCTTCAA	ATAMACAAAT TATTTGTTTA		CACGACCCC
CCACATARCA	CCACTCCTGC	GGCGACACGG CCGCTGTGCC	ATTTAGAMAA TAMATCTTTT		ATACCCCATA TATCCCCATA
TANTACCOCO	TCCATCITAAC AGCTACATIO	AGGGNATAAG TCCCTTATTC	ATTTOAATOT TAAACTTACA		ACCTATAAAA
CAACACGGGA	<b>GAGATCCAGT</b> CTCTAGGTCA	GCCCCAAAA	GCGGATACAT CGCCTATOTA		CATCACA
37001	37101	37201	17301		37401

Figure 15X



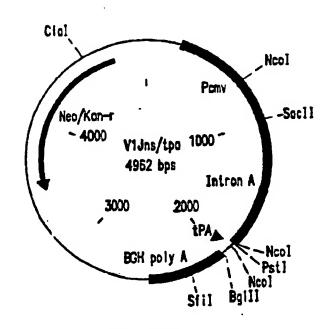


FIGURE 16

	ATTGAGACTGTGCCTGTGAAGCTGAAGCC 1 leG i uThrVo1ProVo1LysLeuLysPr 10	
	CAACGCCCTGGTGGAAATCTGCACTGAGA eLysaidLeuVoiGiulieCysThrGiuW 40	
	CCCCTGTGTTTGCCATCAAGAAGAAGAA hrProVolPheAlolleLysLysLysAsp O	
	CAGGACTTCTGGGAGGTGCAGCTGGGCAT GInAspPheTrpGIuVoIGInLeuGIyII 90	
	GGGGGATGCCTACTTCTCTGTGCCCCTGC IG1yAspA1oTyrPheSerVo1ProLeuA 120	
loPheInrlleProSerlleAsnAsnG	AGACCCCTGGCATCAGGTACCAGTACAA IuThrProGlylleArgTyrGlnTyrAsn 40	
	ACCAAGATCCTGGAGCCCTTCAGGAAGC/ ThrLys1ieLeuGiuProPheArgLysG 170	
	TGACCTGGAGATTGGGCAGCACAGGACC rAspLeuGlulleGlyGlnHisArgThrl 200	
	ACAAGAAGCACCAGAAGGAGCCCCCCTTO splyslysHisGInlysGIuProProPho 120	
	GTGCTGCCTGAGAAGGACTCCTGGACTG ValleuProClulysAspSerTrpThrV 250	
CAAGCTGAACTGGGCCTCCCAAATCTA yLysLeuAsnTrpAloSerGInlleTy 270	CCCTGGCATCAAGGTGAGGCAGCTGTGC rProGiylieLysVolargGinLeuCys 280	AAGCTGCTGAGGGCCACCAAGGCCX LysLeuLeuArgGiyThrLysAiol 290

FIGURE 17A

GGGGTGTACTATGACCCCTCCAAGGACCTGATTGCTGACATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAAATCTA GlyVolTyrTyrAspProSerLysAspLeulleAloGlulleGlnLysGlnGlyGlnGlyGlnTrpThrTyrGlnlleTy 320 330 340

CCADGAGCCCTTCAAGAACCTGAAGACTGCCAAGTATGCCAGGATGAGGGGGGCCCCACACCAATGATGTGAAGCAGCTGA rGInGluProPheLysAsnLeuLysThrGlyLysTyrAlaArgMelArgGlyAlaHisThrAsnAspVoilysGInLeuT 350 350 370

CTCAGGCTGTGCAGAAGATCACCACTGAGTCCATTGTGATCTGGGGCAAGACCCCCAAGTTCAAGCTGCCCATCCAGAAG hrGluAloVolGinLyslleThrThrGluSerlleVollleTrpGlyLysThrProLysPheLysLeuProlleGinLys 380 390

GGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCCATTGTGGGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG uVollysLeuTrpTyrCinLeuGiuLysGiuProlleVolGlyAloGiuThrPheTyrVolAloGlyAloAloAsnArgG 430 440 450

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCTCCCAGTATGC LysThr AloLeuGInAlolleTyrLeuAloLeuGInAspSerGlyLeuGluVolAsnIleVolThr AloSerGInTyrAl 480 490 500

CCTGGGCATCATCCAGGCCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG aleuGiyiielleGinAioGinProAspGinSerGiuSerGiuLeuVolAsnGinIielleGiuGinLeuIielysLysG 510 520 530

ACAAGGTGTACCTGCCCTGCGTGCCTGCCCACAAGGCCATTGGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC
lulysvoltyrleualotrpvolproalohislysglylleglyglyasngluginvolasplysleuvolSeralogly
540 550

ATCAGGAACGTGCTGTTCCTGGATGGCATTGACAACCCCCAGGATGAGCATGAGAAGTACCACTCCAACTGGAGGCCTAT
11eArgLysVolleuPheleuAspGlyI1eAspLysAloGlnAspGluHisGluLysTyrHisSerAsnTrpArgAloMe
560 570 580

#### FIGURE 17B

GGCCTCTGACTTCAACCTGCCCCCTGTGGTGGCTAACGAGATTGTGCCCTCCTGTGACAAGTGCCAGCTGAAGCCCCAGG tAloSerAspPheAsnLeuProProVolVolAloLysGiuIleVolAloSerCysAspLysCysGinLeuLysGiyGiuA 590 600 610

CCATGCATGGGCAGGTGGACTGCTCCCCTGGCATCTGGCAGGTGACCCACCTGGAGGGCAAGGTGATCCTGGTG IOMetHisGlyGlnVolAspCysSerProGlylleTrpGlnLeuAloCysThrHisLeuGluGlyLysVollleLeuVol 620 630

GCTGTGCATGTGGCCTCCGGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGCCCAGGAGACTGCCTACTTCCTGCT AlovolHisVolAloSerGlyTyrlleGluAloGluVollleProAloGluThrGlyGlnGluThrAloTyrPheLeuLe 640 650 660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACACTGCCAATGGCTCCAACTTCACTGGGGCCACAGTGAGGGCTG uLysLeuAloGlyArgTrpProVolLysThrIleHisThrAloAsnGlySerAsnPheThrGlyAloThrVolArgAloA 680 690

CCTGCTGGTGGGCTGGCATCAAGCAGGAGTTTGGCATCCCCTACAACCCCCAGTCCCAGGGGGTGGTGGCCTCCATGAAC IoCysTrpTrpAloGly!leLysGInGluPheGly!leProTyrAsnProGInSerGinGlyVolVolAIoSerMelAsn 700 710

AAGGAGCTGAAGAAGATCATTGGGCAGGTGAGGGACCAGGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTCAT LysGluLeuLysLyslielleGlyGInVolArgAspGInAloGluHisLeuLysThrAloVolGlnMeiAloVolPhell 720 730 740

CCACAACTTCAAGAGGAAGGGGGCATCGGGGGCTACTCCGCTGGGGAGAGGATTGTGGACATCATTGCCACAGACATCC
eHisAsnPheLysArgLysGlyGlylleGlyGlyTyrSerAloGlyGluArglleVolAsplleIleAloThrAsplleG
750
760
770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGGTGTACTACAGGGACTCCAGGAACCCCCTGTGG
InThrLysGTuLeuGInLysGInHeThrLysTleGInAsnPheArgValTyrTyrArgAspSerArgAsnProLeuTrp
780
790

AAGGCCCTGCCAAGCTGCTGTGGAAGGCGGAGGCGGCTGTGGTGATCCAGGACAACTCTGACATCAAGCTGGTGCCCAG LysGiyProAioLysLeuLeuTrpLysGiyGiuGiyAioVoiVoiIieGinAspAsnSerAspIieLysVoiVoiProAr 800 820

GAGGAAGGCCAAGATCATCAGGGACTATGGCAAGCAGATGGCTGGGGATGACTGTGGGCCTCCAGGCACGATGAGGACT gArglysAfolysIteIteArgAspTyrGfyLysGfnMetAfoGfyAspAspCysVotAfoSerArgGinAspGtuAspx 830 840 850

AAAGCCCCGGCAGATC" (SEQ ID NO: 3)
Xx Roll (SEQ ID NO: 4)

FIGURE 17C

PCT/US01/28861 WO 02/022080

CCACCOMMATCTCCCCCCCATCTCCCCCATTCAGACTGTCCTGTCAAGCTGAAGCTGCAAGCCTGCAAGCATGCC (within SEO 10 NO: 7)
RoSerGiulleSerAloProlleSerProlleCluthrVolProValLysleudysProGlyMetAspCly (within SEO 10 NO: 8)
-1 2 20

FIGURE 18

```
- ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT
                                                           -42
M
        - ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC GGC TGG TCC
OPT
         . M G G K W S K R S V P G W S
                                                            .14
        - ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT
WT
                                                            -84
        - ACC GTG AGG GAG AGG ATG AGG AGG GCC GAG CCC GCC GCC GAC
OPT
           TVRERMRRAEPAAD
                                                            -28
        - AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA
                                                            -126
WT
        - AGG GTG AGG AGG ACC GAG CCC GCC GCC GTG GGC GTG GGC GCC
OPT
           RVRRTEPAAVGVGA
                                                            -42
        - GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC
                                                            -168
WT
        . GTG TCC AGG GAC CTG GAG AAG CAC GGC GCC ATC ACC TCC TCC
OPT
           V S R D L E K H G A I T S S
                                                            -56
        - AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA
                                                            -210
WT.
        - AAC ACC GCC GCC ACC AAC GCC GAC TGC GCC TGG CTG GAG GCC
OPT
           NTAATNADCAWLE
                                                            -70.
        - CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA
                                                            .252
WI.
          - CÁG GÁG GÁC GÁG GÁG GTG GGC TTC CCC GTG ÁGG CCC CÁG GTG
OPT
           O E D E E V G F P V R P Q V
                                                            -84
        - CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC
                                                            -294
WT
        - CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC
P L R P M T Y K G A V D L S
OPT
                                                            -98
        - CAC TIT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC
                                                            -336
WT
        - CAC TTC CTG AAG GAG AAG GGC CTG GAG GGC CTG ATC CAC
OPT
           H F L K E K G G L E G L I H
                                                            -112
        - TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC
                                                            -378
WT
        - TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC
OPT
         SOKRODILDLWVYH
                                                            -126
        - ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG
                                                            -420
WT
        - ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC
OPT
           TOGYFPDWDNYTPG
                                                            -140
```

FIGURE 19A

WT	- CCA GGA ATC AGA TIT CCA TTG ACC TTT GGA TGG TGC TTC AAG -462	
OPT	- CCC GGC ATC AGG TTC CCC CTG ACC TTC GGC TGG TGC TTC AAG P G I R F P L T F G W C F K -154	
₩T	- CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA -504	
OPT	- CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GCC AAC GAG L V P V E P E K V E E A N E -168	
WT	- GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG .546	
OPT	- GÁC GÁG ÁÁC ÁÁC TỚC CTỔ CTĆ CÁC CĆC ÁTỔ TCC CÁG CÁC GGC G E N N C L L H P M S Q H G -182	
ਘਾ	- ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC -588	
OPT	- ATC GÁG GÁC CĆC GÁG ÁÁG GÁG GTG CTG GÁG TGG ÁGG TTC GAC I E D P E K E V L E W R F D -196	
WT .	- AGC AAG CTA GCA TTT CAT CAC GTG GCC CGA GAG CTG CAT CCG -630	
OPT	- TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC S K L A F H H V A R E L H P -210	
WT	- GAG TAC TAC AAG GAC TGC TGA (SEQ ID NO:30) -651	
OPT	- GAG TAC TAC AAG GAC TGC TAA (contained within SEQID NO:9) E Y Y K D C (SEQID NO:10) -216	,

FIGURE 19B

VIJns/nef

CATGGGTCTTTT<u>CIGCAG</u>TCACCGTCCTTC<u>AGATC</u>TGCCACC ATG GGC GGC ANG TGG TCC ANG AGG TCC GTG CCC M G G K W S K R S V P

Srf1 Bg111

CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGCACACAICTGCCTTCTAGTTGCCAGC (SEQ 1D NU: 38)

H P E Y Y K D C \* (contained within SEQ 1D NO: 10:

V1Jns/nef(G2A.LLAA)

**Psti** Catibastictiticigasicaccosticticaccacc atg gcc ggc ang tgg tcc ang agg tcc gtg ccc . M A G K W S K R S V P

Srff Bg111

CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGGAGAICIGCTGTGCCTTCTAATTGCCAGC (SEQ 1D NO: 39)

H P E Y Y K D C \* (contained within SEQ 1D NO:14)

Vijns/tpanef & Vijns/tpanef(LLAA)

Psti Catgastettticigasteacceteatatatetagaeacce atg gat gca atg ang aga ctc tgc tgt gtg m D a m k r g l c c v

CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC  $\frac{BgJIJ}{AG}$  ICC TCC AAG AGG TCC GTG CCC

SrfI Bg111

CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGAGAICIGCTGGCCTTCTAGTTGCCAGC (SEQ ID NO: 40)

H P E Y Y K D C \* (confaints) withon stq id No: 16)

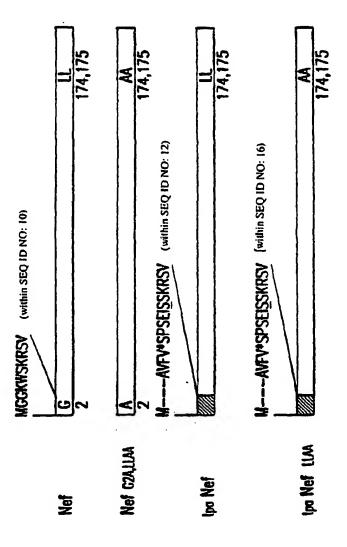


FIGURE 21

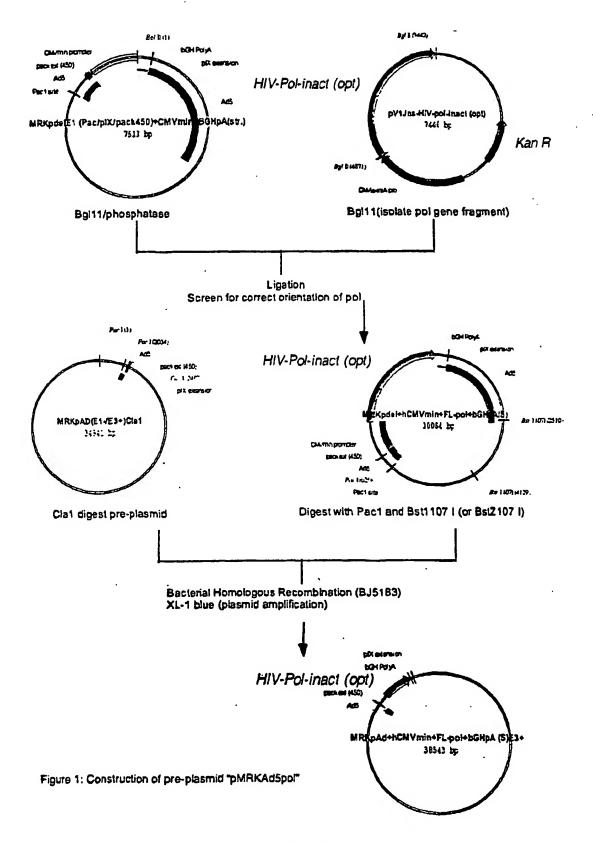
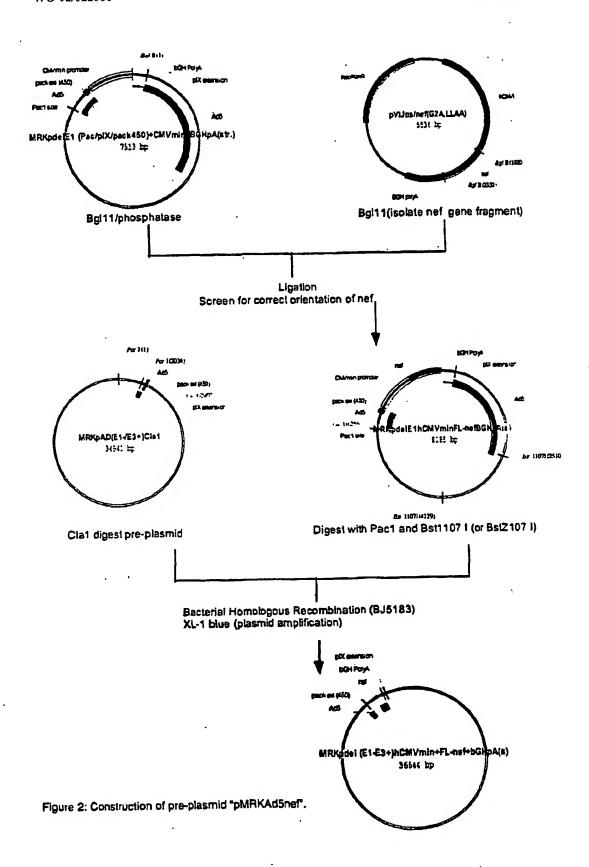
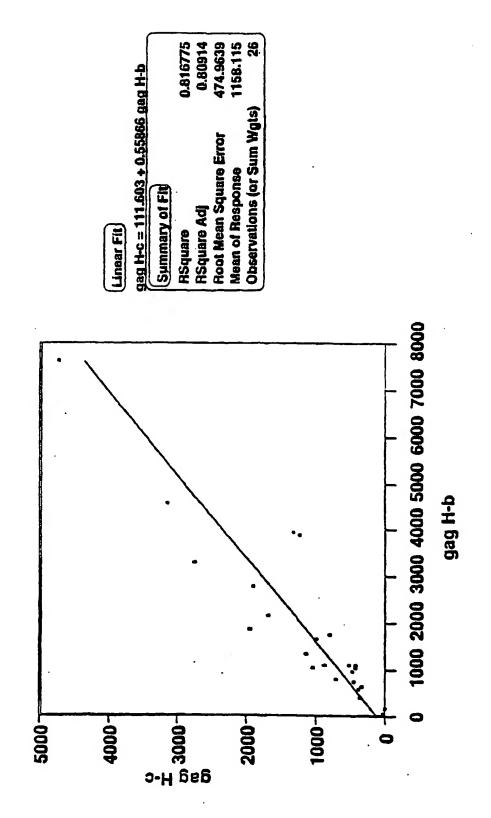


FIGURE 22



Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects



0.91685

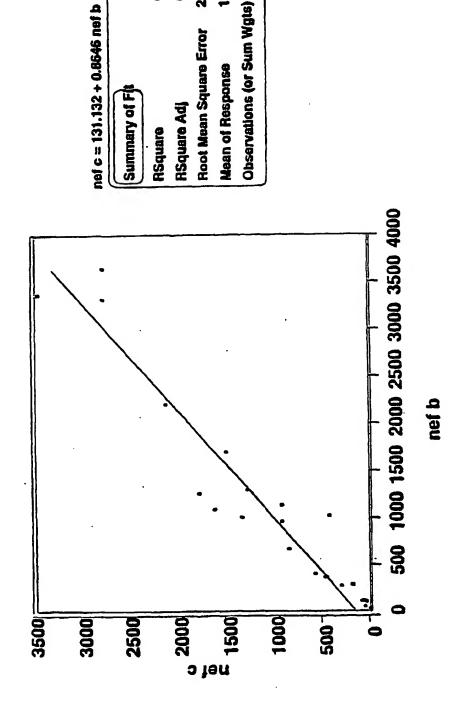
289.7718

FIGURE 25

1096.435

8

Comparison of Clade B vs. Clade C Anti-nef T Cell Responses in Clade B HIV-Infected Subjects

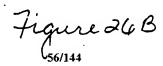


#### MRKAd5pol MER1062 (MRKAd5 Pre-Adenoviral Vector Containing the IA opt pol Coding Region)

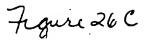
1	CATCATCAAT	AATATACCTT	ATTTTGGATT	GAAGCCAATA	TGATAATGAG
		TTATATGGAA			
51	GGGGTGGAGT	TTCTCACCTC	GCGCGCGCC	TEECAACECE	CCCCCTCACC
7-		AACACTGCAC			
	CCCCACCICA	MACAC 16CAC	CGCGCCCCGC	ACCCTTGCCC	CGCCCACTGC
• • •	#1 C#C#C				
101		GCGGAAGTGT			
	ATCATCACAC	CGCCTTCACA	CTACAACGTT	CACACCGCCT	TGTGTACATT
151	GCGACGGATG	TGGCAAAAGT	GACGTTTTTG	GTGTGCGCCG	GTGTACACAG
	CGCTGCCTAC	ACCGTTTTCA	CTGCAAAAAC	CACACGCGGC	CACATGTGTC
201	GAAGTGACAA	TTTTCGCGCG	GTTTTAGGCG	GATGTTGTAG	TAXATTTGGG
	CTTCACTGTT	AAAAGCGCGC	CAAAATCCGC	CTACAACATC	ATTTAAACCC
251	CGTAACCGAG	TAAGATTTGG	CCATTTTCCC	GCGAAAACTG	AATAACACCA
		ATTCTAAACC			
	ocn1100c1C	MILCIMENCE	GGIMPPIGCG	CCCITITUMC	IIMITETEE
301	A CONCA A A SOCIO	GAATAATTTT	CIDCINIA CIDCA	macccccmaa	mammomoma a
201					
	ICACITIAGA	CTTATTAAAA	CACAATGAGT	ATCGCGCATT	ATAAACAGAT
351		GACTTTGACC			
	CCCGGCGCCC	CTGAAACTGG	CAAATGCACC	TCTGAGCGGG	TCCACAAAAA
401	CTCAGGTGTT	TTCCGCGTTC	CGGGTCAAAG	TTGGCGTTTT	ATTATTATAG
	GAGTCCACAA	AAGGCGCAAG	GCCCAGTTTC	AACCGCAAAA	TAATAATATC
451	GCGGCCGCGA	TCCATTGCAT	ACGITGTATC	CATATCATAA	TATGTACATT
	CGCCGGCGCT	AGGTAACGTA	TGCAACATAG	GTATAGTATT	ATACATGTAA
501	TATATTGGCT	CATGTCCAAC	ATTACCGCCA	TGTTGACATT	GATTATTGAC
		GTACAGGTTG			
551	<b>ጥልርጥጥልጥጥል</b> ል	TAGTAATCAA	TT NCCCCCTC	NAMES CARACTER	NCCCCN TRATA
		ATCATTAGTT			
	VI-TWVIVVII	AICAIIAGII	MIGCCCCNG	IMMICAMOIM	TCGGGTATAT
601	maas ammaaa	000000000000000000000000000000000000000	00000000000	<b>********</b>	Bacamas 666
901		CGTTACATAA			
	ACCICAAGGC	GCAATGTATT	GAATGCCATT	TACCGGGCGG	ACCGACTGGC
651		CCCGCCCATT			
	GGGTTGCTGG	GGGCGGGTAA	CTGCAGTTAT	TACTGCATAC	aagggtatca
			•		
	AACGCCAATA				
	TTGCGGTTAT	CCCTGAAAGG	TAACTGCAGT	TACCCACCTC	ATAAATGCCA
751	AAACTGCCCA	CTTGGCAGTA	CATCAAGTGT	ATCATATGCC	AAGTACGCCC
	TTTGACGGGT	GAACCGTCAT	GTAGTTCACA	TAGTATACGG	TTCATGCGGG
801	CCTATTGACG	TCAATGACGG	TARATGCCC	CCTCCCATT	ATGCCCACTA
		AGTTACTGCC			
	~~		* 2/10000	CGGUCCG1WV	***************************************
ÖE1	CATGACCTTA	JULICA CANTON	CTACTACCA	CONT. C. S. MCDP. C.	CMAMMACMCA
031					
	GIALIGUAAT	ACCCTGAAAG	GATGAACCGT	CATGTAGATG	CATAATCAGT

7 i jure 26A

O ULIULL					
901				AGTACATCAA	
	AGCGATAATG	GTACCACTAC	GCCAAAACCG	TCATGTAGTT	ACCCGCACCT
951	TAGCGGTTTG	ACTCACGGGG	ATTTCCAAGT	CTCCACCCCA	TTGACGTCAA
•••				GAGGTGGGGT	
1001	TGGGAGTTTG	TTTTGGCACC	AAAATCAACG	GGACTTTCCA	AAATGTCGTA
				CCTGAAAGGT	
1051				GTAGGCGTGT	
				CATCCGCACA	
1101				CGTCAGATCG	
				GCAGTCTAGC	
1151				ACACCGGGAC	
				TGTGGCCCTG	
1201				GGATTCCCCG	
				CCTAAGGGGC	
1251				TGAGACTGTG	
				ACTCTGACAC	
1301				AGCAGTGGCC	
				TCGTCACCGG	
1351				ACTGAGATGG	
				TGACTCTACC	
1401				CTACAACACC	
				GATGTTGTGG	
1451				GGAAGCTGGT	
				CCTTCGACCA	
1501				GAGGTGCAGC	
				CTCCACGTCG	
1551	CCACCCCGCT	GGCCTGAAGA	AGAAGAAGTC	TGTGACTGTG	CTGGCTGTGG
				ACACTGACAC	
1601	GGGATGCCTA	CTTCTCTGTG	CCCCTGGATG	AGGACTICAG	GAAGTACACT
					CTTCATGTGA
1651	GCCTTCACCA	TCCCCTCCAT	CAACAATGAG	ACCCCTGGCA	TCAGGTACCA
				TGGGGACCGT	
1701	GTACAATGTG	CTGCCCCAGG	GCTGGAAGGG	CTCCCCTGCC	ATCTTCCAGT
					TAGAAGGTCA
1751	CCTCCATGAC	CAAGATCCTG	GAGCCCTTCA	GGAAGCAGAA	CCCTGACATT
	GGAGGTACTG				
1801	GTGATCTACC	AGTACATGGC	TGCCCTGTAT	GTGGGCTCTG	ACCTGGAGAT
	CACTAGATGG	TCATGTACCG	ACGGGACATA	CACCCGAGAC	TGGACCTCTA



1901			AAGAAGCACC TTCTTCGTGG		
1951			CCCCGACAAG GGGGCTGTTC		
2001			GGACTGTGAA CCTGACACTT		
2051			CAAATCTACC GTTTAGATGG		
2101			CACCAAGGCC GTGGTTCCGG		
2151			AGCTGGCTGA TCGACCGACT		
2201			TATGACCCCT ATACTGGGGA		
2251			CCAGTGGACC GGTCACCTGG		
2301		-	GCAAGTATGC CGTTCATACG		
2351			ACTGAGGCTG TGACTCCGAC		
2401			GACCCCCAAG CTGGGGGTTC		
2451	GGAGACCTGG	GAGACCTGGT	GGACTGAGTA CCTGACTCAT	CTGGCAGGCC	ACCTGGATCC
2501	CTGAGTGGGA	GTTTGTGAAC	ACCCCCCCC TGGGGGGGGG	TGGTGAAGCT	GTGGTACCAG
2551	CTGGAGAAGG	AGCCCATTGT	GGGGGCTGAG CCCCCGACTC	ACCTTCTATG	TGGCTGGGGC
2601	TGCCAACAGG	GAGACCAAGC		TGGCTATGTG	ACCAACAGGG
2651	GCAGGCAGAA	GGTGGTGACC	CTGACTGACA	CCACCAACCA	GAAGACTGCC
2701	CTCCAGGCCA	TCTACCTGGC		TCTGGCCTGG	AGGTGAACAT
2751	TGTGACTGCC		GGAGGTCCTG CCCTGGGCAT		
			GGGACCCGTA		



2851	GAGAAGGTGT	ACCTGGCCTG	GGTGCCTGCC	CACAAGGGCA	TTGGGGGCAA
	CTCTTCCACA	TGGACCGGAC	CCACGGACGG	GTGTTCCCGT	AACCCCCGTT
2901	TGAGCAGGTG	GACAAGCTGG	TGTCTGCTGG	CATCAGGAAG	GTGCTGTTCC
	ACTCGTCCAC	CTGTTCGACC	ACAGACGACC	GTAGTCCTTC	CACGACAAGG
2951	TGGATGGCAT	TGACAAGGCC	CAGGATGAGC	ATGAGAAGTA	CCACTCCAAC
	ACCTACCGTA	ACTGTTCCGG	GTCCTACTCG	TACTCTTCAT	GGTGAGGTTG
3001	TGGAGGGCTA	TGGCCTCTGA	CTTCAACCTG	CCCCTGTGG	TGGCTAAGGA
	ACCTCCCGAT	ACCGGAGACT	GAAGTTGGAC	GGGGGACACC	ACCGATTCCT
3051	CTAACACCGG	AGGACACTGT	AGTGCCAGCT TCACGGTCGA	CTTCCCCCTC	CGGTACGTAC
3101	GGCAGGTGGA	CTGCTCCCCT	GGCATCTGGC	AGCTGGCCTG	CACCCACCTG
	CCGTCCACCT	GACGAGGGGA	CCGTAGACCG	TCGACCGGAC	GTGGGTGGAC
3151	GAGGGCAAGG	TGATCCTGGT	GGCTGTGCAT	GTGGCCTCCG	GCTACATTGA
	CTCCCGTTCC	ACTAGGACCA	CCGACACGTA	CACCGGAGGC	CGATGTAACT
3201	GGCTGAGGTG	ATCCCTGCTG	AGACAGGCCA	GGAGACTGCC	TACTTCCTGC
	CCGACTCCAC	TAGGGACGAC	TCTGTCCGGT	CCTCTGACGG	ATGAAGGACG
3251	TGAAGCTGGC	TGGCAGGTGG	CCTGTGAAGA	CCATCCACAC	TGCCAATGGC
	ACTTCGACCG	ACCGTCCACC	GGACACTTCT	GGTAGGTGTG	ACGGTTACCG
3301	TCCAACTTCA	CTGGGGCCAC	AGTGAGGGCT	GCCTGCTGGT	GGGCTGGCAT
	AGGTTGAAGT	GACCCCGGTG	TCACTCCCGA	CGGACGACCA	CCCGACCGTA
3351	CAAGCAGGAG	TTTGGCATCC	CCTACAACCC	CCAGTCCCAG	GGGGTGGTGG
	GTTCGTCCTC	AAACCGTAGG	GGATGTTGGG	GGTCAGGGTC	CCCCACCACC
3401		GTTCCTCGAC	TTCTTCTAGT	AACCCGTCCA	CTCCCTGGTC
3451	CGACTCGTGG	ACTTCTGTCG	TGTGCAGATG ACACGTCTAC	CGACACAAGT	AGGTGTTGAA
3501	GTTCTCCTTC	CCCCCGTAGC	GGGGCTACTC	GCGACCCCTC	TCCTAACACC
		GTGTCTGTAG	GTCTGGTTCC	TCGAGGTCTT	CGTCTAGTGG
	_	TGAAGTCCCA	CATGATGTCC	CTGAGGTCCT	TGGGGGACAC
		CGGTTCGACG	ACACCTTCCC	CCTCCCCGA	· CACCACTAGG
3701	AGGACAACTC	TGACATCAAG	GTGGTGCCCA	GGAGGAAGGC	CAAGATCATC
	TCCTGTTGAG	ACTGTAGTTC	CACCACGGGT	CCTCCTTCCG	GTTCTAGTAG

7 jure 26 D

3801		TAAAGCCCGG ATTTCGGGCC		
3851	•	TTGCCCCTCC AACGGGGAGG		
3901.		TCCTTTCCTA AGGAAAGGAT		
3951	•	CATTCTATTC GTAAGATAAG		
4001		GGAAGACAAT CCTTCTGTTA		
4051		GGCGCGCGCA		
4101		TATAAGGTGG ATATTCCACC		
4151		GCCGCCATGA CGGCGGTACT		
4201	•	GACAACGCGC CTGTTGCGCG	 	
4251		CCAGCATTGA GGTCGTAACT		
4301		TACGAGACCG ATGCTCTGGC		
4351		TTCAGCCGCT AAGTCGGCGA		
4401		TGAGCCCGCT ACTCGGGCGA	 	-
4451		AAGTTGACGG TTCAACTGCC		
4501		TGTCGTTTCT ACAGCAAAGA		
4551		AGGCTTCCTC TCCGAAGGAG		
4601		TCTGTTTGGA AGACAAACCT		
4651		TTTGCGCGCG AAACGCGCGC		

Figure 26E

PCT/US01/28861 WO 02/022080

4751	GTTCAGATAC	ATGGGCATAA	GCCCGTCTCT	GGGGTGGAGG	TAGCACCACT
	CAAGTCTATG	TACCCGTATT	CGGGCAGAGA	CCCCACCTCC	ATCGTGGTGA
4801	GCAGAGCTTC	ATGCTGCGGG	GTGGTGTTGT	AGATGATCCA	GTCGTAGCAG
	CGTCTCGAAG	TACGACGCCC	CACCACAACA	TCTACTAGGT	CAGCATCGTC
4851	GAGCGCTGGG	CGTGGTGCCT	AAAAATGTCT	TTCAGTAGCA	AGCTGATTGC
	CTCGCGACCC	GCACCACGGA	TTTTTACAGA	AAGTCATCGT	TCGACTAACG
4901	CAGGGGCAGG	CCCTTGGTGT	AAGTGTTTAC	AAAGCGGTTA	AGCTGGGATG
	GTCCCCGTCC	GGGAACCACA	TTCACAAATG	TTTCGCCAAT	TCGACCCTAC
4951	GGTGCATACG	TGGGGATATG	AGATGCATCT	TGGACTGTAT	TTTTAGGTTG
	CCACGTATGC	ACCCCTATAC	TCTACGTAGA	ACCTGACATA	AAAATCCAAC
5001	GCTATGTTCC	CAGCCATATC	CCTCCGGGGA	TTCATGTTGT	GCAGAACCAC
	CGATACAAGG	GTCGGTATAG	GGAGGCCCCT	AAGTACAACA	CGTCTTGGTG
5051	CAGCACAGTG	TATCCGGTGC	ACTTGGGAAA	TTTGTCATGT	AGCTTAGAAG
	GTCGTGTCAC	ATAGGCCACG	TGAACCCTTT	AAACAGTACA	TCGAATCTTC
5101	GAAATGCGTG	GAAGAACTTG	GAGACGCCCT	TGTGACCTCC	AAGATTTTCC
	CTTTACGCAC	CTTCTTGAAC	CTCTGCGGGA	ACACTGGAGG	TTCTAAAAGG
5151	ATGCATTCGT	CCATAATGAT	GGCAATGGGC	CCACGGGCGG	CGGCCTGGGC
	TACGTAAGCA	GGTATTACTA	CCGTTACCCG	GGTGCCCGCC	GCCGGACCCG
5201	GAAGATATTT	CTGGGATCAC	TAACGTCATA	GTTGTGTTCC	AGGATGAGAT
	C'ITCTATAAA	GACCCTAGTG	ATTGCAGTAT	CAACACAAGG	TCCTACTCTA
5251	CGTCATAGGC	CATTTTTACA	AAGCGCGGGC	GGAGGGTGCC	AGACTGCGGT
	GCAGTATCCG	GTAAAAATGT	TTCGCGCCCG	CCTCCCACGG	TCTGACGCCA
5301	ATAATGGTTC	CATCCGGCCC	AGGGGCGTAG	TTACCCTCAC	AGATTTGCAT
	TATTACCAAG	GTAGGCCGGG	TCCCCGCATC	AATGGGAGTG	TCTAAACGTA
5351	TTCCCACGCT	TTGAGTTCAG	ATGGGGGGAT	CATGTCTACC	TGCGGGGCGA
	AAGGGTGCGA	AACTCAAGTC	TACCCCCTA	GTACAGATGG	ACGCCCCGCT
5401	TGAAGAAAAC	GGTTTCCGGG	GTAGGGGAGA	TCAGCTGGGA	AGAAAGCAGG
	ACTTCTTTTG	CCAAAGGCCC	CATCCCCTCT	AGTCGACCCT	TCTTTCGTCC
5451	TTCCTGAGCA	GCTGCGACTT	ACCGCAGCCG	GTGGGCCCGT	AAATCACACC
	AAGGACTCGT	CGACGCTGAA	TGGCGTCGGC	CACCCGGGCA	TTTAGTGTGG
5501	TATTACCGGC	TGCAACTGGT	AGTTAAGAGA	GCTGCAGCTG	CCGTCATCCC
	ATAATGGCCG	ACGTTGACCA	TCAATTCTCT	CGACGTCGAC	GGCAGTAGGG
5551	TGAGCAGGGG	GGCCACTTCG	TTAAGCATGT	CCCTGACTCG	CATGTTTTCC
	ACTCGTCCCC	CCGGTGAAGC	AATTCGTACA	GGGACTGAGC	GTACAAAAGG
5601	CTGACCAAAT	CCGCCAGAAG	GCGCTCGCCG	CCCAGCGATA	GCAGTTCTTG
	GACTGGTTTA	GGCGGTCTTC	CGCGAGCGGC	GGGTCGCTAT	CGTCAAGAAC

Figure 26 F

5701			AGTTCCAGGC TCAAGGTCCG		CTCGGTCACC GAGCCAGTGG
5751			CAGCATATCT GTCGTATAGA		
5801	-		TAGTCGGTGC ATCAGCCACG		
5851	CATGTCTTTC GTACAGAAAG		GGGTCCTCGT CCCAGGAGCA		
5901			TGCGCGCTGG ACGCGCGACC		
5951			CTGCCGGTCT GACGGCCAGA		
6001	CATCGTAAAC	TGGTACCACA	CATAGTCCAG GTATCAGGTC	GGGGAGGCGC	CGCACCGGGA
6091	ACCGCGCGTC	GAACGGGAAC	GAGGAGGCGC CTCCTCCGCG	GCGTGCTCCC	CGTCACGTCT
6101	GAAAACTCCC	GCATCTCGAA	GGGCGCGAGA CCCGCGCTCT	TTATGGCTAA	GGCCCTCAT
6151	CCGTAGGCGC	GGCGTCCGGG	CGCAGACGGT GCGTCTGCCA	GAGCGTAAGG	TGCTCGGTCC
6201	ACTCGAGACC	GGCAAGCCCC	TCAAAAACCA AGTTTTTGGT	CCAAAGGGGG	TACGAAAAAC
6251	TACGCAAAGA	ATGGAGACCA	TTCCATGAGC AAGGTACTCG	GCCACAGGTG	CGAGCCACTG
	CTTTTCCGAC	AGGCACAGGG		GAACTCTCCG	GACAGGAGCT
		CGCCAGGAGG	AGCATATCTT	TGAGCCTGGT	GAGACTCTGT
		AGGTCCGGTC	GTGCTTCCTC	CGATTCACCC	TCCCCATCGC
		TGATCCCCCA	GGTGAGCGAG	GTCCCACACT	TCTGTGTACA
		CCGTAGTTCC	TTCCACTAAC	CAAACATCCA	CATCCGGTGC
6551	TGACCGGGTG ACTGGCCCAC		GGGGCTATAA CCCCGATATT		

Figure 266

6651	AGTACTCCCT	CTGAAAAGCG	GGCATGACTT	CTGCGCTAAG	ATTGTCAGTT
0031	TCATGAGGGA	GACTTTTCGC	CCGTACTGAA	GACGCGATTC	TAACAGTCAA
6701	TCCAAAAACG	AGGAGGATTT	GATATTCACC	TGGCCCGCGG	TGATGCCTTT
	AGGTTTTTGC	TCCTCCTAAA	CTATAAGTGG	ACCGGGCGCC	ACTACGGAAA
6751	GAGGGTGGCC	GCATCCATCT	GGTCAGAAAA	GACAATCTTT	TTGTTGTCAA
			CCAGTCTTTT		
6801	GCTTGGTGGC	AAACGACCCG	TAGAGGGCGT	TGGACAGCAA	CTTGGCGATG
			ATCTCCCGCA		
6851	GAGCGCAGGG	TTTGGTTTTT	GTCGCGATCG	GCGCGCTCCT	TGGCCGCGAT
			CAGCGCTAGC		
6901	GTTTAGCTGC	ACGTATTCGC	GCGCAACGCA	CCGCCATTCG	CCTTTCTCCC
			CGCGTTGCGT		
6951	TGGTGCGCTC	GTCGGGCACC	AGGTGCACGC	GCCAACCGCG	GTTGTGCAGG
			TCCACGTGCG		
7001	GTGACAAGGT	CAACGCTGGT	GGCTACCTCT	CCGCGTAGGC	GCTCGTTGGT
			CCGATGGAGA		
7051	CCAGCAGAGG	CGGCCGCCCT	TGCGCGAGCA	GAATGGCGGT	AGGGGGTCTA
			ACGCGCTCGT		
7101	GCTGCGTCTC	GTCCGGGGGG	TCTGCGTCCA	CGGTAAAGAC	CCCGGGCAGC
			AGACGCAGGT		·
7151	AGGCGCGCGT	CGAAGTAGTC	TATCTTGCAT	CCTTGCAAGT	CTAGCGCCTG
			ATAGAACGTA		
7201	CTGCCATGCG	CGGGCGGCAA	GCGCGCGCTC	GTATGGGTTG	AGTGGGGGAC
	-		CGCGCGCGAG		
7251	CCCATGGCAT	GGGGTGGGTG	AGCGCGGAGG	CGTACATGCC	CCTTTACAGC
			TCGCGCCTCC		
7301	TAAACGTAGA	GGGGCTCTCT	GAGTATTCCA	AGATATGTAG	GGTAGCATCT
					CCATCGTAGA
7351	TCCACCGCGG	ATGCTGGCGC	GCACGTAATC	GTATAGTTCG	TGCGAGGGAG
					ACGCTCCCTC
7401	CGAGGAGGTC	GGGACCGAGG	TTGCTACGGG	CGGGCTGCTC	TGCTCGGAAG
					ACGAGCCTTC
7451	ACTATCTGCC	TGAAGATGGC	ATGTGAGTTG	GATGATATGG	TTGGACGCTG
	TGATAGACGG	ACTTCTACCG	TACACTCAAC	CTACTATACC	AACCTGCGAC
7501	GAAGACGTTG	AAGCTGGCGT	CTGTGAGACC	TACCGCGTCA	CGCACGAAGG
	CTTCTGCAAC	TTCGACCGCA	GACACTCTGG	ATGGCGCAGI	GCGTGCTTCC

Figure 26 H

7601					ACTTATCCTG
7651					TCGCGGTCTT
	AGGGAAAAAA	AAGGTGTCG	GCGCCAACT	C CTGTTTGAG	A AGCGCCAGAA
7701					GTAAGAGCCT CATTCTCGGA
7751					CCTTTTCTAC GGAAAAGATG
7801					TGGGTGAGCG ACCCACTCGC
7851					GAAGTCAGTG CTTCAGTCAC
7901					GCTTTTTGGA CGAAAAACCT
7951					ATCTTTCCCG TAGAAAGGGC
8001					CACCTCGGAA GTGGAGCCTT
8051					AGCCGTTGAT TCGGCAACTA
8101				GCGCGGGATG CGCGCCCTAC	
8151				GCTCTTCAGG CGAGAAGTCC	
8201				TGAGGGTTGG ACTCCCAACC	
8251				TTGCAGGTGG AACGTCCACC	
8301	TCCTAAACTG AGGATTTGAC				
8351	GTAAGCGGGT CATTCGCCCA			CCAAGGTTCG GGTTCCAAGC	
8401	TCGCGCGGCA AGCGCGCCGT				
8451	TGAAGGGCAC ACTTCCCGTG				

Figure 26I

PCT/US01/28861 WO 02/022080

8551	GAAGAACTGG	ATCTCCCGCC	ACCAATTGGA	GGAGTGGCTA	TTGATGTGGT
				CCTCACCGAT	
8601	GAAAGTAGAA	GTCCCTGCGA	CGGGCCGAAC	ACTCGTGCTG	GCTTTTGTAA
5001	CTTTCATCTT	CAGGGACGCT	GCCCGGCTTG	TGAGCACGAC	CGAAAACATT
8651	AAACGTGCGC	AGTACTGGCA	GCGGTGCACG	GGCTGTACAT	CCTGCACGAG
0031	TTTGCACGCG	TCATGACCGT	CGCCACGTGC	CCGACATGTA	GGACGTGCTC
8701	GTTGACCTGA	CGACCGCGCA	CAAGGAAGCA	GAGTGGGAAT	TTGAGCCCCT
0,02	CAACTGGACT	GCTGGCGCGT	GTTCCTTCGT	CTCACCCTTA	AACTCGGGGA
8751	CCCTCCCG	GTTTGGCTGG	TGGTCTTCTA	CTTCGGCTGC	TTGTCCTTGA
0,32	GCGGACCGCC	CAAACCGACC	ACCAGAAGAT	GAAGCCGACG	AACAGGAACT
8801	CCGTCTGGCT	GCTCGAGGGG	<b>AGTTACGGTG</b>	GATCGGACCA	CCACGCCGCG
	GGCAGACCGA	CGAGCTCCCC	TCAATGCCAC	CTAGCCTGGT	GGTGCGGCGC
8851	CGAGCCCAAA	GTCCAGATGT	CCGCGCGCGG	CGGTCGGAGC	TTGATGACAA
•••	GCTCGGGTTT	CAGGTCTACA	GGCGCGCGCC	GCCAGCCTCG	AACTACTGTT
8901	CATCGCGCAG	ATGGGAGCTG	TCCATGGTCT	GGAGCTCCCG	CGGCGTCAGG
	GTAGCGCGTC	TACCCTCGAC	AGGTACCAGA	CCTCGAGGGC	GCCGCAGTCC
8951	TCAGGCGGGA	GCTCCTGCAG	GTTTACCTCG	CATAGACGGG	TCAGGGCGCG
0,00	AGTCCGCCCT	CGAGGACGTC	CAAATGGAGC	GTATCTGCCC	AGTCCCGCGC
9001	GGCTAGATCC	AGGTGATACC	TAATTTCCAG	GGGCTGGTTG	GTGGCGGCGT
• • • • • • • • • • • • • • • • • • • •	CCGATCTAGG	TCCACTATGG	ATTAAAGGTC	CCCGACCAAC	CACCGCCGCA
9051	CGATGGCTTG	CAAGAGGCCG	CATCCCCGCG	GCGCGACTAC	GGTACCGCGC
	GCTACCGAAC	GTTCTCCGGC	GTAGGGGCGC	CGCGCTGATG	CCATGGCGCG
9101	GGCGGGCGGT	GGGCCGCGG	GGTGTCCTTG	GATGATGCAT	CTAAAAGCGG
				CTACTACGTA	
9151	TGACGCGGGC	GAGCCCCCGG	AGGTAGGGGG	GGCTCCGGAC	CCGCCGGGAG
				CCGAGGCCTG	
9201	AGGGGGCAGG	GGCACGTCGG	CCCCCCCCCC	GGGCAGGAGC	TGGTGCTGCG
•				CCCGTCCTCG	
9251	CGCGTAGGTT	GCTGGCGAAC	GCGACGACGC	GGCGGTTGAT	CTCCTGAATC
					GAGGACTTAG
9301	TGGCGCCTCT	GCGTGAAGAC	GACGGGCCCG	GTGAGCTTGA	ACCTGAAAGA
					TGGACTTTCT
9351	GAGTTCGACA	GAATCAATTT	CGGTGTCGTT	GACGGCGGCC	TGGCGCAAAA
	CTCAAGCTGT	CTTAGTTAAA	GCCACAGCA	CIGCCGCCGG	ACCGCGTTTT
9401	TCTCCTGCAC	GTCTCCTGAG	TTGTCTTGAT	AGGCGATCTC	GGCCATGAAC
	AGAGGACGT	CAGAGGACTC	AACAGAACTA	A TCCGCTAGAG	CCGGTACTTG

Figure 26. J 64/144

9501				AAGGCGTTGA TTCCGCAACT
9551				TTCGGCATCG AAGCCGTAGC
9601		 		GCCGGGCGAA CGGCCCGCTT
9651			GTAGTTGAGG CATCAACTCC	GTGGTGGCGG CACCACCGCC
9701		·	AGCGTCGCAA TCGCAGCGTT	CGTGGATTCG GCACCTAAGC
9751			ATGGCCTCGT TACCGGAGCA	
9801		 	CGACACGGTT GCTGTGCCAA	
9851		 	CGCGCACCTC GCGCGTGGAG	
9901		 	TCCTCTTCCA AGGAGAAGGT	
9951			AGGGGGGACA TCCCCCTGT	
10001			GCTCGATCAT CGAGCTAGTA	
10051			CCGTTCTCGC GGCAAGAGCG	
10101			ATGGGTTGGC TACCCAACCG	
10151		 	ATCTCAACAA TAGAGTTGTT	
10201	GGTACTCCGC CCATGAGGCG			
10251	AAACCTCTCG TTTGGAGAGC			
10301	GCACCGTGGC CGTGGCACCG			
10351	GTGCTGCTGA CACGACGACT		<del>-</del>	

Figure 26 K

10451	CGGCCATGCC	CCAGGCTTCG	TTTTGACATC	GGCGCAGGTC	TTTGTAGTAG
	GCCGGTACGG	GGTCCGAAGC	AAAACTGTAG	CCGCGTCCAG	AAACATCATC
10501	TCTTGCATGA	GCCTTTCTAC	CGGCACTTCT	TCTTCTCCTT	CCTCTTGTCC
	AGAACGTACT	CGGAAAGATG	GCCGTGAAGA	AGAAGAGGAA	GGAGAACAGG
10551	TGCATCTCTT	GCATCTATCG	CTGCGGCGGC	GGCGGAGTTT	GGCCGTAGGT
	ACGTAGAGAA	CGTAGATAGC	GACGCCGCCG	CCGCCTCAAA	CCGGCATCCA
10601	GGCGCCCTCT	TCCTCCCATG	CGTGTGACCC	CGAAGCCCCT	CATCGGCTGA
	CCGCGGGAGA	AGGAGGGTAC	GCACACTGGG	GCTTCGGGGA	GTAGCCGACT
10651	AGCAGGGCTA	GGTCGGCGAC	AACGCGCTCG	GCTAATATGG	CCTGCTGCAC
	TCGTCCCGAT	CCAGCCGCTG	TTGCGCGAGC	CGATTATACC	GGACGACGTG
10701	CTGCGTGAGG	GTAGACTGGA	AGTCATCCAT	GTCCACAAAG	CGGTGGTATG
	GACGCACTCC	CATCTGACCT	TCAGTAGGTA	CAGGTGTTTC	GCCACCATAC
10751	CGCCCGTGTT	GATGGTGTAA	GTGCAGTTGG	CCATAACGGA	CCAGTTAACG
	GCGGGCACAA	CTACCACATT	CACGTCAACC	GGTATTGCCT	GGTCAATTGC
10801	GTCTGGTGAC	CCGGCTGCGA	GAGCTCGGTG	TACCTGAGAC	GCGAGTAAGC
	CAGACCACTG	GGCCGACGCT	CTCGAGCCAC	ATGGACTCTG	CGCTCATTCG
10851	CCTCGAGTCA	AATACGTAGT	CGTTGCAAGT	CCGCACCAGG	TACTGGTATC
	GGAGCTCAGT	TTATGCATCA	GCAACGTTCA	GGCGTGGTCC	ATGACCATAG
10901	CCACCAAAAA	GTGCGGCGGC	GGCTGGCGGT	AGAGGGGCCA	GCGTAGGGTG
	GGTGGTTTTT	CACGCCGCCG	CCGACCGCCA	TCTCCCCGGT	CGCATCCCAC
10951	GCCGGGGCTC	CGGGGGCGAG	ATCTTCCAAC	ATAAGGCGAT	GATATCCGTA
	CGGCCCCGAG	GCCCCCGCTC	TAGAAGGTTG	TATTCCGCTA	CTATAGGCAT
11001	GATGTACCTG	GACATCCAGG	TGATGCCGGC	GGCGGTGGTG	GAGGCGCGCG
	CTACATGGAC	CTGTAGGTCC	ACTACGGCCG	CCGCCACCAC	CTCCGCGCGC
11051	GAAAGTCGCG	GACGCGGTTC	CAGATGTTGC	GCAGCGGCAA	AAAGTGCTCC
	CTTTCAGCGC	CTGCGCCAAG	GTCTACAACG	CGTCGCCGTT	TTTCACGAGG
11101	ATGGTCGGGA	CGCTCTGGCC	GGTCAGGCGC	GCGCAATCGT	TGACGCTCTA
	TACCAGCCCT	GCGAGACCGG	CCAGTCCGCG	CGCGTTAGCA	ACTGCGAGAT
11151	GACCGTGCAA	AAGGAGAGCC	TGTAAGCGGG	CACTCTTCCG	TGGTCTGGTG
	CTGGCACGTT	TTCCTCTCGG	ACATTCGCCC	GTGAGAAGGC	ACCAGACCAC
	CTATTTAAGC	GTTCCCATAG	TACCGCCTGC	TGGCCCCAAG	GAGCCCCGTA CTCGGGGCAT
	AGGCCGGCAG	GCGGCACTAG	GTACGCCAAT	GGCGGGCGCA	GTCGAACCCA CAGCTTGGGT
11301	GGTGTGCGAC	GTCAGACAAC	GGGGGAGTGC	TCCTTTTGGC	TTCCTTCCAG
	CCACACGCTG	CAGTCTGTTG	CCCCTCACG	AGGAAAACCG	AAGGAAGGTC

Figure 26L

11401			AGTGGCTCGC TCACCGAGCG	
11451			GGGACCCCCG CCCTGGGGGC	
11501			TTGCCTCCCC AACGGAGGGG	
11551			GACGAGCCCC CTGCTCGGGG	
11601			GCGCCCCCT CGCGGGGGGA	
11651			GGGCACCCTC CCCGTGGGAG	
11701			GACGCGGCAG CTGCGCCGTC	
11751			CTACCTGGAC GATGGACCTG	
11801			CTCCTGAGCG GAGGACTCGC	
11851			TACGTGCCGC ATGCACGGCG	
11901			GGAGATGCGG CCTCTACGCC	
11951			TGAATCGCGA ACTTAGCGCT	
12001			ACCGGGATTA TGGCCCTAAT	
12051			CGCATACGAG GCGTATGCTC	
12101	ACCAGGAGAT TGGTCCTCTA	••	 ACAACCACGT TGTTGGTGCA	
12151	GTGGCGCGCG CACCGCGCGC		 ATGCATCTGT TACGTAGACA	
12201	AAGCGCGCTG TTCGCGCGAC	_	 GCCGCTCATG CGGCGAGTAC	
12251	TĊCTTATAGT AGGAATATCA		AGGCATTCAG TCCGTAAGTC	

71 gure 26 M

12351	CCTGCAGAGC	ATAGTGGTGC	AGGAGCGCAG	CTTGAGCCTG GAACTCGGAC	GCTGACAAGG CGACTGTTCC
				TGGGCAAGTT	
12401	ACCGCCGCTA	GTTGATAAGG	TACGAATCGG	ACCCGTTCAA	AATGCGGGCG
12451	AAGATATACC	ATACCCCTTA	CGTTCCCATA	GACAAGGAGG CTGTTCCTCC	TAAAGATCGA ATTTCTAGCT
12501				GCTTACCTTG	
12501	CCCCAAGATG	TACGCGTACC	GCGACTTCCA	CGAATGGAAC	TCGCTGCTGG
12551	TGGGCGTTTA ACCCGCAAAT	TCGCAACGAG AGCGTTGCTC	CGCATCCACA GCGTAGGTGT	AGGCCGTGAG TCCGGCACTC	CGTGAGCCGG GCACTCGGCC
12601	CGGCGCGAGC	TCAGCGACCG	CGAGCTGATG	CACAGCCTGC GTGTCGGACG	AAAGGGCCCT
				•	•
12651	GGCTGGCACG	CCGTCGCCGC	TATCTCTCCG	CGAGTCCTAC GCTCAGGATG	AAACTGCGCC
12701	GCGCTGACCT CGCGACTGGA	GCGCTGGGCC CGCGACCCGG	CCAAGCCGAC GGTTCGGCTG	GCGCCCTGGA CGCGGGACCT	GGCAGCTGGG CCGTCGACCC
12751	GCCGGACCTG CGGCCTGGAC	GGCTGGCGGT CCGACCGCCA	GGCACCCGCG CCGTGGGCGC	CGCGCTGGCA GCGCGACCGT	ACGTCGGCGG TGCAGCCGCC
12801	CGTGGAGGAA	TATGACGAGG	ACGATGAGTA	CGAGCCAGAG	GACGGCGAGT CTGCCGCTCA
12851	ACTAAGCGGT TGATTCGCCA	GATGTTTCTG CTACAAAGAC	TAGTCTACTA	CGTTCTGCGT	ACGGACCCGG TGCCTGGGCC
12901	CGGTGCGGGC	GGCGCTGCAG	AGCCAGCCGT	CCGGCCTTAA	CTCCACGGAC GAGGTGCCTG
12951	GACTGGCGCC CTGACCGCGG	AGGTCATGGA TCCAGTACCT	CCGCATCATG	AGCGACTGAC	CGCGCAATCC GCGCGTTAGG
13001	TGACGCGTTC ACTGCGCAAG	CGGCAGCAGC GCCGTCGTCG	CGCAGGCCAA GCGTCCGGTT	CCGGCTCTCC	GCAATTCTGG CGTTAAGACC
13051	AAGCGGTGGT TTCGCCACCA	CCCGGCGCGC	GCAAACCCCA CGTTTGGGGT	CGCACGAGAA GCGTGCTCTT	GGTGCTGGCG
13101	ATCGTAAACG TAGCATTTGC	CGCTGGCCGA GCGACCGGCT	AAACAGGGCC TTTGTCCCGG	ATCCGGCCCG	ACGAGGCCGG TGCTCCGGCC
13151	CCTGGTCTAC GGACCAGATG	GACGCGCTGC CTGCGCGACG	TTCAGCGCGT AAGTCGCGCA	GGCTCGTTAC	AACAGCGGCA TTGTCGCCGT
13201	ACGTGCAGAC TGCACGTCTG	CAACCTGGAC GTTGGACCTG	CGGCTGGTGG	GGGATGTGCG	CGAGGCCGTG CCTCCGGCAC

Figure 26 N

13301		TTCCTGAGTA AAGGACTCAT		
13351		CAACTTTGTG GTTGAAACAC		
13401		AGGTGTACCA TCCACATGGT		
13451		CTGCAGACCG GACGTCTGGC		
13501		GGGGGTGCGG CCCCCACGCC		
13551		CGCCCAACTC GCGGGTTGAG		
13601		GGCAGCGTGT CCGTCGCACA		
13651		CGAGGCCATA GCTCCGGTAT		
13701		CAAGTGTCAG GTTCACAGTC		
13751		ACCCTAAACT TGGGATTTGA		
13801		CAGTTTAAAC GTCAAATTTG		
13851		TGAGCCTTAA ACTCGGAATT		
13901		ATGACCGCGC TACTGGCGCG		
13951		TATCAACCGC ATAGTTGGCG		
14001	GTGAACCCCG CACTTGGGGC	AGTATTTCAC TCATAAAGTG		
14051	GCCCCCTGGT CGGGGGACCA	TTCTACACCG AAGATGTGGC		
14101	GATTCCTCTG CTAAGGAGAC	GGACGACATA CCTGCTGTAT		
14151	ACCCTGCTAG TGGGACGATC	AGTTGCAACA TCAACGTTGT	•	

7, gure 260

14251	CGCGGTCAGA	TGCTAGTAGC	CCATTTCCAA	GCTTGATAGG	GTCTCTTACC
	GCGCCAGTCT	ACGATCATCG	GGTAAAGGTT	CGAACTATCC	CAGAGAATGG
14301	AGCACTCGCA	CCACCCGCCC	GCGCCTGCTG	GGCGAGGAGG	AGTACCTAAA
	TCGTGAGCGT	GGTGGGCGGG	CGCGGACGAC	CCGCTCCTCC	TCATGGATTT
14351	CAACTCGCTG	CTGCAGCCGC	AGCGCGAAAA	AAACCTGCCT	CCGGCATTTC
	GTTGAGCGAC	GACGTCGGCG	TCGCGCTTTT	TTTGGACGGA	GGCCGTAAAG
14401	CCAACAACGG	GATAGAGAGC	CTAGTGGACA	AGATGAGTAG	ATGGAAGACG
	GGTTGTTGCC	CTATCTCTCG	GATCACCTGT	TCTACTCATC	TACCTTCTGC
14451	TACGCGCAGG ATGCGCGTCC	AGCACAGGGA TCGTGTCCCT	CGTGCCAGGC GCACGGTCCG	CCGCGCCCGC	CCACCCGTCG GGTGGGCAGC
14501	TCAAAGGCAC	GACCGTCAGC	GGGGTCTGGT	GTGGGAGGAC	GATGACTCGG
	AGTTTCCGTG	CTGGCAGTCG	CCCCAGACCA	CACCCTCCTG	CTACTGAGCC
14551	CAGACGACAG	CAGCGTCCTG	GATTTGGGAG	GGAGTGGCAA	CCCGTTTGCG
	GTCTGCTGTC	GTCGCAGGAC	CTAAACCCTC	CCTCACCGTT	GGGCAAACGC
14601	CACCTTCGCC	CCAGGCTGGG	GAGAATGTTT	TAAAAAAAAA	AAAAGCATGA
	GTGGAAGCGG	GGTCCGACCC	CTCTTACAAA	TTTTTTTTT	TTTTCGTACT
14651	TGCAAAATAA	AAAACTCACC	AAGGCCATGG	CACCGAGCGT	TGGTTTTCTT
	ACGTTTTATT	TTTTGAGTGG	TTCCGGTACC	GTGGCTCGCA	ACCAAAAGAA
14701	GTATTCCCCT CATAAGGGGA	TAGTATGCGG ATCATACGCC	CGCGCGCCGCT	TGTATGAGGA ACATACTCCT	AGGTCCTCCT TCCAGGAGGA
14751	CCCTCCTACG	AGAGTGTGGT	GAGCGCGGCG	CCAGTGGCGG	CGGCGCTGGG
	GGGAGGATGC	TCTCACACCA	CTCGCGCCGC	GGTCACCGCC	GCCGCGACCC
14801	TTCTCCCTTC AAGAGGGAAG	GATGCTCCCC CTACGAGGGG	TGGACCCGCC ACCTGGGCGG	GTTTGTGCCT CAAACACĢGA	CCGCGGTACC
14851	TGCGGCCTAC	CGGGGGGAGA	AACAGCATCC	GTTACTCTGA	GTTGGCACCC
	ACGCCGGATG	GCCCCCTCT	TTGTCGTAGG	CAATGAGACT	CAACCGTGGG
14901	CTATTCGACA	CCACCCGTGT	GTACCTGGTG	GACAACAAGT	CAACGGATGT
	GATAAGCTGT	GGTGGGCACA	CATGGACCAC	CTGTTGTTCA	GTTGCCTACA
14951	GGCATCCCTG	AACTACCAGA	ACGACCACAG	CAACTTTCTG	ACCACGGTCA
	CCGTAGGGAC	TTGATGGTCT	TGCTGGTGTC	GTTGAAAGAC	TGGTGCCAGT
15001	TTCAAAACAA AAGTTTTGTT	TGACTACAGC ACTGATGTCG	CCGGGGGAGG	CAAGCACACA GTTCGTGTGT	GACCATCAAT CTGGTAGTTA
15051	CTTGACGACC GAACTGCTGG	GGTCGCACTG CCAGCGTGAC	GGGCGGCGAC	CTGAAAACCA GACTTTTGGT	TCCTGCATAC AGGACGTATG
15101	CAACATGCCA	AATGTGAACG	AGTTCATGTT	TACCAATAAG	TTTAAGGCGC
	GTTGTACGGT	TTACACTTGC	TCAAGTACAA	ATGGTTATTC	AAATTCCGCG

Figure 26 P

WO 02/022080 PCT/US01/28861 15151 GGGTGATGGT GTCGCGCTTG CCTACTAAGG ACAATCAGGT GGAGCTGAAA CCCACTACCA CAGCGCGAAC GGATGATTCC TGTTAGTCCA CCTCGACTTT 15201 TACGAGTGGG TGGAGTTCAC GCTGCCCGAG GGCAACTACT CCGAGACCAT ATGCTCACCC ACCTCAAGTG CGACGGGCTC CCGTTGATGA GGCTCTGGTA 15251 GACCATAGAC CTTATGAACA ACGCGATCGT GGAGCACTAC TTGAAAGTGG CTGGTATCTG GAATACTTGT TGCGCTAGCA CCTCGTGATG AACTTTCACC 15301 GCAGACAGAA CGGGGTTCTG GAAAGCGACA TCGGGGTAAA GTTTGACACC CGTCTGTCTT GCCCCAAGAC CTTTCGCTGT AGCCCCATTT CAAACTGTGG 15351 CGCAACTTCA GACTGGGGTT TGACCCCGTC ACTGGTCTTG TCATGCCTGG GCGTTGAAGT CTGACCCCAA ACTGGGGCAG TGACCAGAAC AGTACGGACC 15401 GGTATATACA AACGAAGCCT TCCATCCAGA CATCATTTTG CTGCCAGGAT CCATATATGT TTGCTTCGGA AGGTAGGTCT GTAGTAAAAC GACGGTCCTA 15451 GCGGGGTGGA CTTCACCCAC AGCCGCCTGA GCAACTTGTT GGGCATCCGC CGCCCACCT GAAGTGGGTG TCGGCGGACT CGTTGAACAA CCCGTAGGCG 15501 AAGCGGCAAC CCTTCCAGGA GGGCTTTAGG ATCACCTACG ATGATCTGGA TTCGCCGTTG GGAAGGTCCT CCCGAAATCC TAGTGGATGC TACTAGACCT 15551 GGGTGGTAAC ATTCCCGCAC TGTTGGATGT GGACGCCTAC CAGGCGAGCT CCCACCATTG TAAGGGCGTG ACAACCTACA CCTGCGGATG GTCCGCTCGA 15601 TGAAAGATGA CACCGAACAG GGCGGGGGTG GCGCAGGCGG CAGCAACAGC ACTITICIACT GIGGCITGIC CCGCCCCCAC CGCGTCCGCC GICGITGICG 15651 AGTGGCAGCG GCGCGGAAGA GAACTCCAAC GCGGCAGCCG CGGCAATGCA TCACCGTCGC CGCGCCTTCT CTTGAGGTTG CGCCGTCGGC GCCGTTACGT 15701 GCCGGTGGAG GACATGAACG ATCATGCCAT TCGCGGCGAC ACCTTTGCCA CGGCCACCTC CTGTACTTGC TAGTACGGTA AGCGCCGCTG TGGAAACGGT 15751 CACGGGCTGA GGAGAAGCGC GCTGAGGCCG AAGCAGCGGC CGAAGCTGCC GTGCCCGACT CCTCTTCGCG CGACTCCGGC TTCGTCGCCG GCTTCGACGG 15801 GCCCCGCTG CGCAACCCGA GGTCGAGAAG CCTCAGAAGA AACCGGTGAT CGGGGGCGAC GCGTTGGGCT CCAGCTCTTC GGAGTCTTCT TTGGCCACTA 15851 CANACCCCTG ACAGAGGACA GCAAGAAACG CAGTTACAAC CTAATAAGCA GTTTGGGGAC TGTCTCCTGT CGTTCTTTGC GTCAATGTTG GATTATTCGT 15901 ATGACAGCAC CTTCACCCAG TACCGCAGCT GGTACCTTGC ATACAACTAC TACTGTCGTG GAAGTGGGTC ATGCCGTCGA CCATGGAACG TATGTTGATG 15951 GGCGACCCTC AGACCGGAAT CCGCTCATGG ACCCTGCTTT GCACTCCTGA CCGCTGGGAG TCTGGCCTTA GGCGAGTACC TGGGACGAAA CGTGAGGACT 16001 CGTAACCTGC GGCTCGGAGC AGGTCTACTG GTCGTTGCCA GACATGATGC GCATTGGACG CCGAGCCTCG TCCAGATGAC CAGCAACGGT CTGTACTACG 16051 AAGACCCCGT GACCTTCCGC TCCACGCGCC AGATCAGCAA CTTTCCGGTG

Figure 26 Q

TTCTGGGGCA CTGGAAGGCG AGGTGCGCGG TCTAGTCGTT GAAAGGCCAC

16151	GGCCGTCTAC	TCCCAACTCA	TCCGCCAGTT	TACCTCTCTG	ACCCACGTGT
	CCGGCAGATG	AGGGTTGAGT	AGGCGGTCAA	ATGGAGAGAC	TGGGTGCACA
16201	TCAATCGCTT AGTTAGCGAA	TCCCGAGAAC AGGGCTCTTG	CAGATTTTGG GTCTAAAACC	CGCGCCCGCC	AGCCCCCACC TCGGGGGTGG
16251	ATCACCACCG	TCAGTGAAAA	CGTTCCTGCT	CTCACAGATC	ACGGGACGCT
	TAGTGGTGGC	AGTCACTTTT	GCAAGGACGA	GAGTGTCTAG	TGCCCTGCGA
16301	ACCGCTGCGC	AACAGCATCG	GAGGAGTCCA	GCGAGTGACC	ATTACTGACG
	TGGCGACGCG	TTGTCGTAGC	CTCCTCAGGT	CGCTCACTGG	TAATGACTGC
16351	GGTCTGCGGC	GTGGACGGGG	TACGTTTACA ATGCAAATGT	TCCGGGACCC	GTATCAGAGC
16401	CCGCGCGTCC	TATCGAGCCG	CACTTTTTGA	GCAAGCATGT	CCATCCTTAT
	GGCGCGCAGG	ATAGCTCGGC	GTGAAAAACT	CGTTCGTACA	GGTAGGAATA
16451	ATCGCCCAGC	AATAACACAG	GCTGGGGCCT	GCGCTTCCCA	AGCAAGATGT
	TAGCGGGTCG	TTATTGTGTC	CGACCCCGGA	CGCGAAGGGT	TCGTTCTACA
16501	TTGGCGGGGC	CAAGAAGCGC	TCCGACCAAC	ACCCAGTGCG	CGTGCGCGGG
	AACCGCCCCG	GTTCTTCGCG	AGGCTGGTTG	TGGGTCACGC	GCACGCGCCC
16551	CACTACCGCG	CGCCCTGGGG	CGCGCACAAA	CGCGGCCGCA	CTGGGCGCAC
	GTGATGGCGC	GCGGGACCCC	GCGCGTGTTT	GCGCCGGCGT	GACCCGCGTG
16601	CACCGTCGAT	GACGCCATCG	ACGCGGTGGT	GGAGGAGGCG	CGCAACTACA
	GTGGCAGCTA	CTGCGGTAGC	TGCGCCACCA	CCTCCTCCGC	GCGTTGATGT
16651	CGCCCACGCC	GCCACCAGTG	TCCACAGTGG	ACGCGGCCAT	TCAGACCGTG
	GCGGGTGCGG	CGGTGGTCAC	AGGTGTCACC	TGCGCCGGTA	AGTCTGGCAC
16701	GTGCGCGGAG	CCCGGCGCTA	TGCTAAAATG	AAGAGACGGC	GGAGGCGCGT
	CACGCGCCTC	GGGCCGCGAT	ACGATTTTAC	TTCTCTGCCG	CCTCCGCGCA
16751	AGCACGTCGC TCGTGCAGCG	CACCGCCGCC GTGGCGGCGG	GACCCGGCAC CTGGGCCGTG	TGCCGCCCAA ACGGCGGGTT	CGCGCGCGC
16801	CGGCCCTGCT	TAACCGCGCA	CGTCGCACCG	GCCGACGGGC	GGCCATGCGG
	GCCGGGACGA	ATTGGCGCGT	GCAGCGTGGC	CGGCTGCCCG	CCGGTACGCC
16851	GCCGCTCGAA	GGCTGGCCGC	GGGTATTGTC	ACTGTGCCCC	CCAGGTCCAG
	CGGCGAGCTT	CCGACCGGCG	CCCATAACAG	TGACACGGGG	GGTCCAGGTC
16901	GCGACGAGCG	GCCGCCGCAG	CAGCCGCGCC	CATTAGTGCT	ATGACTCAGG
	CGCTGCTCGC	CGGCGGCGTC	GTCGGCGCCCG	GTAATCACGA	TACTGAGTCC
16951	GTCGCAGGGG CAGCGTCCCC	CAACGTGTAT GTTGCACATA	TGGGTGCGCG ACCCACGCGC	ACTCGGTTAG TGAGCCAATC	CGGCCTGCGC
17001	GTGCCCGTGC	GCACCCGCCC	CCCGCGCAAC	TAGATTGCAA	GAAAAAACTA
	CACGGGCACG	CGTGGGCGGG	GGGCGCGTTG	ATCTAACGTT	CTTTTTTGAT

Figure 26R

17101					CATCGCGCCG
	GATACAGGTT	CGCGTTTTAG	TTTCTTCTCT	ACGAGGTCCA	GTAGCGCGGC
17151					AGCCCCGAAA
	CTCTAGATAC	CGGGGGCTT	CTTCCTTCTC	GTCCTAATGT	TCGGGGCTTT
17201		GTCAAAAAGA			
	CGATTTCGCC	CAGTTTTTCT	TTTTCTTTCT	ACTACTACTA	CTTGAACTGC
17251		ACTGCTGCAC TGACGACGTG			
17301		GCGTAAAACG CGCATTTTGC			
	111000010	CGCATTITGC	ACAMARCUCI	GGGCCG1GG1	GGCATCAGAA
17351		GAGCGCTCCA			
	ATGCGGGCCA	CTCGCGAGGT	GGGCGTGGAT	GTTCGCGCAC	ATACTACTCC
17401	TGTACGGCGA	CGAGGACCTG	CTTGAGCAGG	CCAACGAGCG	CCTCGGGGAG
	ACATGCCGCT	GCTCCTGGAC	GAACTCGTCC	GGTTGCTCGC	GGAGCCCCTC
17451		GAAAGCGGCA			
	AAACGGATGC	CTTTCGCCGT	ATTCCTGTAC	GACCGCAACG	GCGACCTGCT
17501		ACACCTAGCC			
	CCCGTTGGGT	TGTGGATCGG	ATTTCGGGCA	TTGTGACGTC	GTCCACGACG
17551		ACCGTCCGAA			
	GGCGCGAACG	TGGCAGGCTT	CTTTTCGCGC	CGGATTTCGC	GCTCAGACCA
17601		CCACCGTGCA			
	CTGAACCGTG	GGTGGCACGT	CGACTACCAT	GGGTTCGCGG	TCGCTGACCT
17651		GAAAAAATGA			
	TCTACAGAAC	CTTTTTTACT	GGCACCTTGG	ACCCGACCTC	GGGCTCCAGG
17701		AATCAAGCAG			
	CGCACGCCGG	TTAGTTCGTC	CACCGCGGCC	CTGACCCGCA	CGTCTGGCAC
17751		TACCCACTAC			
	CTGCAAGTCT	ATGGGTGATG	GTCATCGTGG	TCATAACGGT	GGCGGTGTCT
17801	GGGCATGGAG				
	CCCGTACCTC	TGTGTTTGCA	GGGGCCAACG	GAGTCGCCAC	CGCCTACGGC
17851	CGGTGCAGGC				
	GCCACGTCCG	CCAGCGACGC	CGGCGCAGGT	TCTGGAGATG	CCTCCACGTT
17901	ACGGACCCGT				
	TGCCTGGGCA	CCTACAAAGC	GCAAAGTCGG	GCCCCCCCC	GCGCGGCAAG
17951	GAGGAAGTAC				
	CTCCTTCATG	CCGCGGCGGT	CGCGCGATGA	CGGGCTTATA	CGGGATGTAG

Figure 265

PCT/US01/28861 WO 02/022080

18051	AGACGAGCAA TCTGCTCGTT	CTACCCGACG GATGGGCTGC	CCGAACCACC GGCTTGGTGG	ACTGGAACCC TGACCTTGGG	CGGCGCCGCCG
18101	TCGCCGTCGC AGCGGCAGCG	CAGCCCGTGC GTCGGGCACG	TGGCCCCGAT ACCGGGGCTA	TTCCGTGCGC AAGGCACGCG	AGGGTGGCTC TCCCACCGAG
18151	GCGAAGGAGG CGCTTCCTCC	CAGGACCCTG GTCCTGGGAC	GTGCTGCCAA CACGACGGTT	CAGCGCGCTA GTCGCGCGAT	CCACCCAGC GGTGGGGTCG
18201	ATCGTTTAAA TAGCAAATTT	AGCCGGTCTT TCGGCCAGAA	TGTGGTTCTT ACACCAAGAA	GCAGATATGG CGTCTATACC	CCCTCACCTG GGGAGTGGAC
18251	GGCGGAGGCA	AAGGGCCACG	CGGGATTCCG GCCCTAAGGC	TCCTTCTTAC	GTGGCATCCT
18301	CCCCGTACCG	GCCGGTGCCG	CTGACGGGCG GACTGCCCGC	CGTACGCAGC	ACGCGTGGTG
18351	GCCGCCGCCG	CGCGCAGCGT	CCGTCGCATG GGCAGCGTAC	GCGCCGCCAT	AGGACGGGGA
18401	GGAATAAGGT	GACTAGCGGC	CGGCGATTGG GCCGCTAACC	GCGGCACGGG	CCTTAACGTA
18451	CCGTGGCCTT GGCACCGGAA	GCAGGCGCAG CGTCCGCGTC	AGACACTGAT TCTGTGACTA	TAAAAACAAG ATTTTTGTTC	TTGCATGTGG AACGTACACC
18501	TTTTTAGTTT	TATTTTTCAG	TGGACTCTCA ACCTGAGAGT	GCGAGCGAAC	CAGGACATTG
18551	ATAAAACATC	TTACCTTCTG	ATCAACTTTG TAGTTGAAAC	GCAGAGACCG	GGGCGCTGTG
18601	GGCTCGCGCC CCGAGCGCGG	CGTTCATGGG GCAAGTACCC	AAACTGGCAA TTTGACCGTT	GATATCGGCA CTATAGCCGT	CCAGCAATAT GGTCGTTATA
18651	CTCGCCACCG	CGGAAGTCGA	GGGGCTCGCT CCCCGAGCGA	CACCTCGCCG	TAATTTTTAA
18701	AGCCAAGGTG	GCAATTCTTG		TCCGGACCTT	GTCGTCGTGT
		ACTCCCTATT	CAACTTTCTC	GTTTTAAAGG	TTGTTTTCCA
	•	GACCGGAGAC	CGTAATCGCC	CCACCACCTG	GACCGGTTGG
18851	AGGCAGTGCA TCCGTCACGT	AAATAAGATT TTTATTCTAA	AACAGTAAGC TTGTCATTCG	TTGATCCCCG AACTAGGGGC	CCCTCCCGTA GGGAGGGCAT
18901	GAGGAGCCTC CTCCTCGGAG	CACCGGCCGT GTGGCCGGCA	GGAGACAGTG CCTCTGTCAC	TCTCCAGAGG AGAGGTCTCC	GGCGTGGCGA CCGCACCGCT

Figure 26.T

19001					CACCACCCGT GTGGTGGGCA
19051		CCATGGCTAC GGTACCGATG		•	=
19101	•	CCTCCCCCG GGAGGGGGGC			
19151		CGTTGTTGTA GCAACAACAT			
19201		GTCCGCGATC CAGGCGCTAG			
19251	· <del>-</del> ·	AACAGCATCG TTGTCGTAGC			
19301		CTGATAGCTA GACTATCGAT			
19351		CAGAGGAGCT GTCTCCTCGA			
19401		TTCGATGATG AAGCTACTAC			
19451		CGGAGTACCT GCCTCATGGA			
19501		TACTTCAGCC ATGAAGTCGG			
19551		CGACGTGACC GCTGCACTGG			
19601		TGGACCGTGA ACCTGGCACT			
19651	GTGGGATCGA	GTGGGTGATA CACCCACTAT	TGGCACACGA	CCTGTACCGA	AGGTGCATGA
	AACTGTAGGC	GCCGCACGAC	CTGTCCCCGG	GATGAAAATT	
19751	GGCACTGCCT CCGTGACGGA	ACAACGCCCT TGTTGCGGGA			
		CGACGATGAC	GAGAACTTTA	TTTGGATCTT	CTTCTCCTGC
19851	ATGACAACGA TACTGTTGCT	AGACGAAGTA TCTGCTTCAT			

Figure 26 U

19951	TCAAATAGGT	GTCGAAGGTC	AAACACCTAA	ATATGCCGAT	AAAACATTTC
	AGTTTATCCA	CAGCTTCCAG	TTTGTGGATT	TATACGGCTA	TTTTGTAAAG
20001	AACCTGAACC	TCAAATAGGA	GAATCTCAGT	GGTACGAAAC	AGAAATTAAT
	TTGGACTTGG	AGTTTATCCT	CTTAGAGTCA	CCATGCTTTG	TCTTTAATTA
20051	CATGCAGCTG	GGAGAGTCCT	AAAAAAGACT	ACCCCAATGA	AACCATGTTA
	GTACGTCGAC	CCTCTCAGGA	TTTTTTCTGA	TGGGGTTACT	TTGGTACAAT
20101	CGGTTCATAT	GCAAAACCCA	CAAATGAAAA	TGGAGGGCAA	GGCATTCTTG
	GCCAAGTATA	CGTTTTGGGT	GTTTACTTTT	ACCTCCCGTT	CCGTA-GAAC
20151	TAAAGCAACA	AAATGGAAAG	CTAGAAAGTC	AAGTGGAAAT	GCAATTTTTC
	ATTTCGTTGT	TTTACCTTTC	GATCTTTCAG	TTCACCTTTA	CGTTAAAAAG
20201	TCAACTACTG	AGGCAGCCGC	AGGCAATGGT	GATAACTTGA	CTCCTAAAGT
	AGTTGATGAC	TCCGTCGGCG	TCCGTTACCA	CTATTGAACT	GAGGATTTCA
20251	GGTATTGTAC	AGTGAAGATG	TAGATATAGA	AACCCCAGAC	ACTCATATTT
	CCATAACATG	TCACTTCTAC	ATCTATATCT	TTGGGGTCTG	TGAGTATAAA
20301	CTTACATGCC	CACTATTAAG	GAAGGTAACT	CACGAGAACT	AATGGGCCAA
	GAATGTACGG	GTGATAATTC	CTTCCATTGA	GTGCTCTTGA	TTACCCGGTT
20351	CAATCTATGC	CCAACAGGCC	TAATTACATT	GCTTTTAGGG	ACAATTTTAT
	GTTAGATACG	GGTTGTCCGG	ATTAATGTAA	CGAAAATCCC	TGTTAAAATA
20401	TGGTCTAATG	TATTACAACA	GCACGGGTAA	TATGGGTGTT	CTGGCGGGCC
	ACCAGATTAC	ATAATGTTGT	CGTGCCCATT	ATACCCACAA	GACCGCCCGG
20451	AAGCATCGCA	GTTGAATGCT	GTTGTAGATT	TGCAAGACAG	AAACACAGAG
	TTCGTAGCGT	CAACTTACGA	CAACATCTAA	ACGTTCTGTC	TTTGTGTCTC
20501	CTTTCATACC	AGCTTTTGCT	TGATTCCATT	GGTGATAGAA	CCAGGTACTT
	GAAAGTATGG	TCGAAAACGA	ACTAAGGTAA	CCACTATCTT	GGTCCATGAA
20551	AAGATACACC	AATCAGGCTG TTAGTCCGAC	AACTGTCGAT	ACTAGGTCTA	CAATCTTAAT
20601	AACTTTTAGT	ACCTTGACTT	CTACTTGAAG	GTTTAATGAC	
	CCTCCACACT	AATTATGTCT	CTGAGAATGG	TTCCATTTTG	CTAAAACAGG GATTTTGTCC
	AGTCCTTTTA	CCTACCCTTT	TTCTACGATG	TCTTAAAAGT	GATAAAAATG CTATTTTTAC
	TTTATTCTCA	ACCTTTATTA	AAACGGTACC	TTTAGTTAGA	AAATGCCAAC TTTACGGTTG
20801	CTGTGGAGAA GACACCTCTT	ATTTCCTGTA TAAAGGACAT	CTCCAACATA GAGGTTGTAT	GCGCTGTATT	TGCCCGACAA ACGGGCTGTT

Tigure 26 V

20901		CCGGGCTAGT GGCCCGATCA	
20951		TATATGGACA ATATACCTGT	
21001		CTACCGCTCA GATGGCGAGT	
21051		AGGTGCCTCA TCCACGGAGT	
21101		TCATACACCT AGTATGTGGA	
21151		GAGCTCCCTA CTCGAGGGAT	
21201		ATAGCATTTG TATCGTAAAC	
21251		TCCACGCTTG AGGTGCGAAC	
21301		CGACTATCTC GCTGATAGAG	
21351		CCAACGTGCC GGTTGCACGG	
21401		TGGGCCTTCA ACCCGGAAGT	
21451		CTACGACCCT GATGCTGGGA	
21501		CCTTTTACCT GGAAAATGGA	
21551		TCTGTCAGCT AGACAGTCGA	
21601		AATTAAGCGC TTAATTCGCG	
21651		TGACCAAAGA ACTGGTTTCT	
21701		 TACCAGGGCT ATGGTCCCGA	
21751		 CTTTAGAAAC GAAATCTTTG	

Figure 26 W

21851	GGCATCCTAC	ACCAACACAA	CAACTCTGGA	TTTGTTGGCT	ACCTTGCCCC
	CCGTAGGATG	TGGTTGTGTT	GTTGAGACCT	AAACAACCGA	TGGAACGGGG
21901				TAACTTCCCC ATTGAAGGGG	
21951	TAGGCAAGAC	CGCAGTTGAC	AGCATTACCC	AGAAAAAGTT	TCTTTGCGAT
	ATCCGTTCTG	GCGTCAACTG	TCGTAATGGG	TCTTTTTCAA	AGAAACGCTA
22001	CGCACCCTTT	GGCGCATCCC	ATTCTCCAGT	AACTTTATGT	CCATGGGCGC
	GCGTGGGAAA	CCGCGTAGGG	TAAGAGGTCA	TTGAAATACA	GGTACCCGCG
22051	ACTCACAGAC	CTGGGCCAAA	ACCTTCTCTA	CGCCAACTCC	GCCCACGCGC
	TGAGTGTCTG	GACCCGGTTT	TGGAAGAGAT	GCGGTTGAGG	CGGGTGCGCG
22101	TAGACATGAC	TTTTGAGGTG	GATCCCATGG	ACGAGCCCAC	CCTTCTTTAT
	ATCTGTACTG	AAAACTCCAC	CTAGGGTACC	TGCTCGGGTG	GGAAGAAATA
22151	GTTTTGTTTG	AAGTCTTTGA	CGTGGTCCGT	GTGCACCAGC	CGCACCGCGG
	CAAAACAAAC	TTCAGAAACT	GCACCAGGCA	CACGTGGTCG	GCGTGGCGCC
22201	CGTCATCGAA	ACCGTGTACC	TGCGCACGCC	CTTCTCGGCC	GGCAACGCCA
	GCAGTAGCTT	TGGCACATGG	ACGCGTGCGG	GAAGAGCCGG	CCGTTGCGGT
22251	CAACATAAAG	AAGCAAGCAA	CATCAACAAC	AGCTGCCGCC	ATGGGCTCCA
	GTTGTATTTC	TTCGTTCGTT	GTAGTTGTTG	TCGACGGCGG	TACCCGAGGT
22301	GTGAGCAGGA	ACTGAAAGCC	ATTGTCAAAG	ATCTTGGTTG	TGGGCCATAT
	CACTCGTCCT	TGACTTTCGG	TAACAGTTTC	TAGAACCAAC	ACCCGGTATA
22351	TTTTTGGGCA	CCTATGACAA	GCGCTTTCCA	GGCTTTGTTT	CTCCACACAA
	AAAAACCCGT	GGATACTGTT	CGCGAAAGGT	CCGAAACAAA	GAGGTGTGTT
22401	GCTCGCCTGC	GCCATAGTCA	ATACGGCCGG	TCGCGAGACT	GGGGGCGTAC
	CGAGCGGACG	CGGTATCAGT	TATGCCGGCC	AGCGCTCTGA	CCCCCGCATG
22451	ACTGGATGGC	CTTTGCCTGG	AACCCGCACT	CAAAAACATG	CTACCTCTTT
	TGACCTACCG	GAAACGGACC	TTGGGCGTGA	GTTTTTGTAC	GATGGAGAAA
22501	GAGCCCTTTG	GCTTTTCTGA	CCAGCGACTC	AAGCAGGTTT	ACCAGTTTGA
	CTCGGGAAAC	CGAAAAGACT	GGTCGCTGAG	TTCGTCCAAA	TGGTCAAACT
22551	GTACGAGTCA	CTCCTGCGCC	GTAGCGCCAT	TGCTTCTTCC	CCCGACCGCT
	CATGCTCAGT	GAGGACGCGG	CATCGCGGTA	ACGAAGAAGG	GGGCTGGCGA
22601	GTATAACGCT	GGAAAAGTCC	ACCCAAAGCG	TACAGGGGCC	CAACTCGGCC
	CATATTGCGA	CCTTTTCAGG	TGGGTTTCGC	ATGTCCCCGG	GTTGAGCCGG
22651	GCCTGTGGAC	TATTCTGCTG	CATGTTTCTC	CACGCCTTTG	CCAACTGGCC
	CGGACACCTG	ATAAGACGAC	GTACAAAGAG	GTGCGGAAAC	GGTTGACCGG
22701	CCAAACTCCC	ATGGATCACA	ACCCCACCAT	GAACCTTATT	ACCGGGGTAC
	GGTTTGAGGG	TACCTAGTGT	TGGGGTGGTA	CTTGGAATAA	TGGCCCCATG

Figure 26 X

22801	••••		CCTGGAGCGC GGACCTCGCG		
22851			GCGCCACTTC CGCGGTGAAG		
22901			GACACTTTCA CTGTGAAAGT		
22951			ATTTACCCC TAAATGGGGG		
23001			GCCGCGCATC CGGCGCGTAG		
23051			TTAGTGCTCC AATCACGAGG		
23101			GTTTTCACTC CAAAAGTGAG		
23151			GCGCCGATAT CGCGGCTATA		
23201	GAGGCGGGAC	GCGCGCGCTC	TTGCGATACA AACGCTATGT	GTCCCAACGT	CGTGACCTTG
23251	TGATAGTCGC	GGCCCACCAC	CACGCTGGCC GTGCGACCGG	TCGTGCGAGA	ACAGCCTCTA
23301	GTCTAGGCGC	AGGTCCAGGA	CCGCGTTGCT GGCGCAACGA	GTCCCGCTTG	CCTCAGTTGA
23351	AACCATCGAC	GGAAGGGTTT	AAGGGCGCGT TTCCCGCGCA	CGGGTCCGAA	ACTCAACGTG
23401	AGCGTGGCAT	CACCGTAGTT	AAGGTGACCG TTCCACTGGC	ACGGGCCAGA	CCCGCAATCC
23451	TATGTCGCGG	ACGTATTTTC	•	GAATTTTCGG	.TGGACTCGGA
•	TTGCGCCTTC AACGCGGAAG	TCTCTTCTTG	TACGGCGTTC	TGAACGGCCT	TTTGACTAAC
	-	GGCGCAGCAC	GTGCGTCGTG	GAACGCAGCC	ACAACCTCTA
		AAAGCCGGGG	TGGCCAAGAA	GTGCTAGAAC	CGGAACGATC
23651	ACTGCTCCTT TGACGAGGAA	CAGCGCGCGC GTCGCGCGCG	TGCCCGTTTT ACGGGCAAAA	CGCTCGTCAC GCGAGCAGTG	ATCCATTTCA TAGGTAAAGT

Figure 26 Y

02/022080					PCT/US01/28861
22701	ATCACGTGCT		ር አጥአ አጥርርጥጥ	CCGTGTAGAC	እርጥጥል አርርጥር
23701				GGCACATCTG	
23751	GCCTTCGATC	TCAGCGCAGC	GGTGCAGCCA	CAACGCGCAG	CCCGTGGGCT
	CGGAAGCTAG	AGTCGCGTCG	CCACGTCGGT	GTTGCGCGTC	GGGCACCCGA
23801	CGTGATGCTT	GTAGGTCACC	TCTGCAAACG	ACTGCAGGTA	CGCCTGCAGG
	GCACTACGAA	CATCCAGTGG	AGACGTTTGC	TGACGTCCAT	GCGGACGTCC
23851	AATCGCCCCA				
	TTAGCGGGGT	AGTAGCAGTG	TTTCCAGAAC	AACGACCACT	TCCAGTCGAC
23901	CAACCCGCGG	TGCTCCTCGT	TCAGCCAGGT	CTTGCATACG	GCCGCCAGAG
23701				GAACGTATGC	
	G17666C6CC	АСОЛООЛОСЛ	Adicodicca	Grand Control	0000001010
23951	CTTCCACTTG	GTCAGGCAGT	AGTTTGAAGT	TCGCCTTTAG	ATCGTTATCC
				AGCGGAAATC	
					. •
24001	ACGTGGTACT	TGTCCATCAG	CGCGCGCGCA	GCCTCCATGC	CCTTCTCCCA
••	TGCACCATGA	ACAGGTAGTC	GCGCGCGCGT	CGGAGGTACG	GGAAGAGGGT
				a	1 mmm
24051				CATCACCGTA	
	GCGTCTGTGC	TAGCCGTGTG	AGTCGCCCAA	GTAGTGGCAT	TAAAGTGAAA
24101	ССССТТСССТ	CCCCTCTTCC	<b>ጥር</b> ጥጥር ርጥር ጥጥ	GCGTCCGCAT	ACCACGCGCC
24101				CGCAGGCGTA	
	GGCGAAGCGA	CCCGAGAAGG	AGAAGGAGAA	CGCAGGCGTA	100100000
24151	ACTGGGTCGT	CTTCATTCAG	CCGCCGCACT	GTGCGCTTAC	CTCCTTTGCC
				CACGCGAATG	
24201	ATGCTTGATT	AGCACCGGTG	GGTTGCTGAA	ACCCACCATT	TGTAGCGCCA
	TACGAACTAA	TCGTGGCCAC	CCAACGACTT	TGGGTGGTAA	ACATCGCGGT
24251				TTACCTCTGG	
	GTAGAAGAGA	AAGAAGGAGC	GACAGGTGCT	AATGGAGACC	ACTACCGCCC
24301	CGCTCGGGCT	TGGGAGAAGG	GCGCTTCTTT	TTCTTCTTGG	GCGCAATGGC
24501				AAGAAGAACC	
	GCGAGCCCGA	Acceletice			
24351	CAAATCCGCC	GCCGAGGTCG	ATGGCCGCGG	GCTGGGTGTG	CGCGGCACCA
	GTTTAGGCGG	CGGCTCCAGC	TACCGGCGCC	CGACCCACAC	GCGCCGTGGT
•	•				
24401	GCGCGTCTTG	TGATGAGTCT	TCCTCGTCCT	CGGACTCGAT	ACGCCGCCTC
	CGCGCAGAAC	ACTACTCAGA	AGGAGCAGGA	GCCTGAGCTA	TGCGGCGGAG
04453	ATCCGCTTTT	mm00000000	COCCCCACCC	CCCCCCCACC	CCCACCCCA
24451					
	TAGGCGAAAA	AACCCCCGCG	GGCCCCTCCG	CCGCCGCTGC	CCCIGCCCCT
24501	CGACACGTCC	TCCATGGTTG	GGGGACGTCG	CGCCGCACCG	CGTCCGCGCT
				GCGGCGTGGC	
	ac 1 G 1 GC 1 GG				
24551	CGGGGGTGGT				
	GCCCCCACCA	AAGCGCGACG	AGGAGAAGGG	CTGACCGGTA	AAGGAAGAGG
24601	TATAGGCAGA				
	ATATCCGTCT	TTTTCTAGTA	CCTCAGTCAG	CTCTTCTTCC	TGTCGGATTG

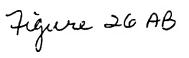
wo

Figure 262

24701		CCCCGTCGAG GGGGCAGCTC		
24751		ACCCAGGTTT TGGGTCCAAA		
24801		GATAAAAAGC CTATTTTTCG		
24851		GCGGGGGAC CGCCCCCTG		
24901		TGTTGAAGCA ACAACTTCGT		
24951		GAGCGCAGCG CTCGCGTCGC	 	
25001		ACGCCACCTA TGCGGTGGAT		
25051		CATGCGAGCC GTACGCTCGG		
25101		GAGGTGCTTG CTCCACGAAC		
25151		ATCCTGCCGT TAGGACGGCA		
25201		AGGGCGCTGT TCCCGCGACA		
25251	•	TTTGAGGGTC AAACTCCCAG		
25301		GGAAAACAGC CCTTTTGTCG		
25351		GTGACAACGC CACTGTTGCG	 	
25401	GGTCACCCAC CCAGTGGGTG	TTTGCCTACC AAACGGATGG	 	
25,451	GCACAGTCAT CGTGTCAGTA	GAGTGAGCTG CTCACTCGAC		
25501	GATGCAAATT CTACGTTTAA			
25551	CGAGCAGCTA GCTCGTCGAT	GCGCGCTGGC CGCGCGACCG		

7 igure 26 AA

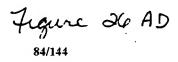
25651	TGCATGCAGC ACGTACGTCG	GGTTCTTTGC CCAAGAAACG	TGACCCGGAG ACTGGGCCTC	ATGCAGCGCA TACGTCGCGT	AGCTAGAGGA TCGATCTCCT
25701				CGTACGCCAG GCATGCGGTC	
25751	TCTCCAACGT AGAGGTTGCA	GGAGCTCTGC CCTCGAGACG	AACCTGGTCT TTGGACCAGA	CCTACCTTGG GGATGGAACC	AATTTTGCAC TTAAAACGTG
25801	GAAAACCGCC CTTTTGGCGG	TTGGGCAAAA AACCCGTTTT	CGTGCTTCAT GCACGAAGTA	TCCACGCTCA AGGTGCGAGT	AGGGCGAGGC TCCCGCTCCG
25851	GCGCCGCGAC CGCGGCGCTG	TACGTCCGCG ATGCAGGCGC	ACTGCGTTTA TGACGCAAAT	CTTATTTCTA GAATAAAGAT	TGCTACACCT ACGATGTGGA
25901	CCGTCTGCCG	GTACCCGCAA	ACCGTCGTCA	GCTTGGAGGA CGAACCTCCT	CACGTTGGAG
25951	AAGGAGCTGC TTCCTCGACG	AGAAACTGCT TCTTTGACGA	AAAGCAAAAC TTTCGTTTTG	TTGAAGGACC AACTTCCTGG	TATGGACGGC ATACCTGCCG
26001	GAAGTTGCTC	GCGAGGCACC	GGCGCGTGGA	GGCGGACATC CCGCCTGTAG	TAAAAGGGGC
26051	TTGCGGACGA	ATTTTGGGAC	GTTGTCCCAG	TGCCAGACTT ACGGTCTGAA	GTGGTCAGTT
26101	TCGTACAACG	TCTTGAAATC	CTTGAAATAG	CTAGAGCGCT GATCTCGCGA	GTCCTTAGAA
26151	CGGGCGGTGG	ACGACACGTG	AAGGATCGCT	CTTTGTGCCC GAAACACGGG	TAATTCATGG
26201	CGCTTACGGG	AGGCGGCGAA	ACCCCGGTGA	GCTACCTTCT CGATGGAAGA	CGTCGATCGG
26251	TTGATGGAAC	GGATGGTGAG	ACTGTATTAC	GAAGACGTGA CTTCTGCACT	CGCCACTGCC
26301	AGATGACCTC	ACAGTGACAG	CGACGTTGGA	ATGCACCCCG TACGTGGGGC	GTGGCGAGGG
		AAGCGTCGAC	GAATTGCTTT	CAGTTTAATA	GCCATGGAAA
		CAGGGAGCGG	ACTGCTTTTC	AGGCGCCGAG	GCCCCAACTT
	ACTCACTCCG TGAGTGAGGC	CCCGACACCT	GCAGCCGAAT	GGAAGCGTTT	AAACATGGAC
26501	AGGACTACCA TCCTGATGGT	CGCCCACGAG GCGGGTGCTC	ATTAGGTTCT TAATCCAAGA	ACGAAGACCA TGCTTCTGGT	ATCCCGCCCG TAGGGCGGGC



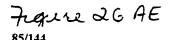
WO 02/022080					PCT/US01/28861
26551	CCTAATGCGG	ACCTTACCCC	<b>CTCCCTCATT</b>	ACCCAGGGCC	ACATTCTTGG
20331	• • • • • • • • • •			TGGGTCCCGG	
26601	CCAATTGCAA	GCCATCAACA	AAGCCCGCCA	AGAGTTTCTG	CTACGAAAGG
	GGTTAACGTT	CGGTAGTTGT	TTCGGGCGGT	TCTCAAAGAC	GATGCTTTCC
26651	GACGGGGGGT	TTACTTGGAC	CCCCAGTCCG	GCGAGGAGCT	CAACCCAATC
				CGCTCCTCGA	
26701	CCCCGCCGC				
	GCGGCCGCCG	GCGTCGGGAT	AGTCGTCGTC	GCGCCCGGG	AACGAAGGGT
26751	GGATGGCACC	CAAAAAGAAG	CTGCAGCTGC	CGCCGCCACC	CACGGACGAG
				GCGGCGGTGG	
26801	GAGGAATACT				
				CAAAACCTGC	
26851	GGACATGATG				
				GCTCCTTCGA	
26901	AAGAGGTGTC			GCCAGCGTAA	
26051	GCGCCCCAGA				
26931				TACCGATGTT	
27001	TCAGGCGCCG				
27001				TGGGTTGGCA	
27051	CCACTGGAAC	CAGGGCCGGT	AAGTCCAAGC	AGCCGCCGCC	GTTAGCCCAA
	GGTGACCTTG	GTCCCGGCCA	TTCAGGTTCG	TCGGCGGCGG	CAATCGGGTT
27101	GAGCAACAAC	AGCGCCAAGG	CTACCGCTCA	TGGCGCGGGC	ACAAGAACGC
	•			ACCGCGCCCG	
27151	CATAGTTGCT				
				GTTGTAGAGG	
27201	GCTTTCTTCT				
27251	TACTACCGTC				GTAGGACGTA .
	ATGATGGCAG				
27301	CAGCAGCGGC	CACACAGAAG	CAAAGGCGAC	CGGATAGCAA	GACTCTGACA
	GTCGTCGCCG	GTGTGTCTTC	GTTTCCGCTG	GCCTATCGTT	CTGAGACTGT
27351	AAGCCCAAGA				
	TTCGGGTTCT		•		
	TCTGGCGCCC				
	AGACCGCGGG				
	TTCCCACTCT				
	AAGGGTGAGA	CATACGATAT	AAAGTTGTCT	CGTCCCCGGT	TUTTGTTCTC

Tiguri 26 AC 83/144

27551	TCACAAAAGC AGTGTTTTCG	GAAGATCAGC CTTCTAGTCG	TTCGGCGCAC AAGCCGCGTG	GCTGGAAGAC CGACCTTCTG	GCGGAGGCTC CGCCTCCGAG
27601	TCTTCAGTAA AGAAGTCATT	ATACTGCGCG TATGACGCGC	CTGACTCTTA GACTGAGAAT	AGGACTAGTT TCCTGATCAA	TCGCGCCCTT AGCGCGGGAA
27651	TCTCAAATTT AGAGTTTAAA	AAGCGCGAAA TTCGCGCTTT	ACTACGTCAT TGATGCAGTA	CTCCAGCGGC GAGGTCGCCG	CACACCCGGC GTGTGGGCCG
27701	GCCAGCACCT CGGTCGTGGA	GTTGTCAGCG CAACAGTCGC	CCATTATGAG GGTAATACTC	CAAGGAAATT GTTCCTTTAA	CCCACGCCCT GGGTGCGGGA
27751	ACATGTGGAG TGTACACCTC	TTACCAGCCA AATGGTCGGT	CAAATGGGAC GTTTACCCTG	TTGCGGCTGG AACGCCGACC	AGCTGCCCAA TCGACGGGTT
27801	CTGATGAGTT	CCCGAATAAA GGGCTTATTT	GATGTACTCG	CGCCCTGGGG	TGTACTATAG
27851	GGCCCAGTTG	GGAATACGCG CCTTATGCGC	GGGTGGCTTT	GGCTTAAGAG	GACCTTGTCC
27901	GCCGATAATG	CACCACACCT GTGGTGTGGA	GCATTATTGG	AATTAGGGGC	ATCAACCGGG
27951	CGACGGGACC	TGTACCAGGA ACATGGTCCT	TTCAGGGCGA	GGGTGGTGAC	ACCATGAAGG
28001	GTCTCTGCGG	CAGGCCGAAG GTCCGGCTTC	AAGTCTACTG	ATTGAGTCCC	CGCGTCGAAC
28051	GCCCGCCGAA	TCGTCACAGG AGCAGTGTCC	CACGCCAGCG	GGCCCGTCCC	ATATTGAGTG
28101	GACTGTTAGT	GAGGGCGAGG CTCCCGCTCC	ATAAGTCGAG	TTGCTGCTCA	GCCACTCGAG
28151	GAGCGAACCA	GAGGCAGGCC	TGCCCTGTAA	AGTCTAGCCG	GGCGCCGGCC CCGCGGCCGG
		GTGCGGAGCA	GTCCGTTAGG	ATTGAGACGT	CTGGAGCAGG
	AGACTCGGCG	CGAGACCTCC	GTAACCTTGA	GACGTTAAAT	TTGAGGAGTT AACTCCTCAA
	ACACGGTAGO	CAGATGAAAT	TGGGGAAGAG	CCCTGGAGGG	GGCCACTATC CCGGTGATAG
	GCCTAGTTAA	ATAAGGATTG	AAACTGCGCC	ATTTCCTGAG	GGCGGACGGC CCGCCTGCCG
28401	TACGACTGAA ATGCTGACTT	TGTTAAGTGG ACAATTCACC	: AGAGGCAGAG : TCTCCGTCTC	GTTGACGCGC	TGAAACACCT ACTTTGTGGA



WO 02/022080 PCT/US01/28861 28451 GGTCCACTGT CGCCGCCACA AGTGCTTTGC CCGCGACTCC GGTGAGTTTT CCAGGTGACA GCGGCGGTGT TCACGAAACG GGCGCTGAGG CCACTCAAAA 28501 GCTACTITGA ATTGCCCGAG GATCATATCG AGGGCCCGGC GCACGGCGTC CGATGAAACT TAACGGGCTC CTAGTATAGC TCCCGGGCCG CGTGCCGCAG 28551 CGGCTTACCG CCCAGGGAGA GCTTGCCCGT AGCCTGATTC GGGAGTTTAC GCCGAATGGC GGGTCCCTCT CGAACGGGCA TCGGACTAAG CCCTCAAATG 28601 CCAGCGCCC CTGCTAGTTG AGCGGGACAG GGGACCCTGT GTTCTCACTG GGTCGCGGG GACGATCAAC TCGCCCTGTC CCCTGGGACA CAAGAGTGAC 28651 TGATTTGCAA CTGTCCTAAC CCTGGATTAC ATCAAGATCT TTGTTGCCAT ACTAAACGTT GACAGGATTG GGACCTAATG TAGTTCTAGA AACAACGGTA 28701 CTCTGTGCTG AGTATAATAA ATACAGAAAT TAAAATATAC TGGGGCTCCT GAGACACGAC TCATATTATT TATGTCTTTA ATTTTATATG ACCCCGAGGA 28751 ATCGCCATCC TGTAAACGCC ACCGTCTTCA CCCGCCCAAG CAAACCAAGG TAGCGGTAGG ACATTTGCGG TGGCAGAAGT GGGCGGGTTC GTTTGGTTCC 28801 CGAACCTTAC CTGGTACTTT TAACATCTCT CCCTCTGTGA TTTACAACAG GCTTGGAATG GACCATGAAA ATTGTAGAGA GGGAGACACT AAATGTTGTC 28851 TTTCAACCCA GACGGAGTGA GTCTACGAGA GAACCTCTCC GAGCTCAGCT AAAGTTGGGT CTGCCTCACT CAGATGCTCT CTTGGAGAGG CTCGAGTCGA 28901 ACTCCATCAG AAAAAACACC ACCCTCCTTA CCTGCCGGGA ACGTACGAGT TGAGGTAGTC TTTTTTGTGG TGGGAGGAAT GGACGGCCCT TGCATGCTCA 28951 GCGTCACCGG CCGCTGCACC ACACCTACCG CCTGACCGTA AACCAGACTT CGCAGTGGCC GGCGACGTGG TGTGGATGGC GGACTGGCAT TTGGTCTGAA 29001 TTTCCGGACA GACCTCAATA ACTCTGTTTA CCAGAACAGG AGGTGAGCTT AAAGGCCTGT CTGGAGTTAT TGAGACAAAT GGTCTTGTCC TCCACTCGAA 29051 AGAAAACCCT TAGGGTATTA GGCCAAAGGC GCAGCTACTG TGGGGTTTAT TCTTTTGGGA ATCCCATAAT CCGGTTTCCG CGTCGATGAC ACCCCAAATA 29101 GAACAATTCA AGCAACTCTA CGGGCTATTC TAATTCAGGT TTCTCTAGAA CTTGTTAAGT TCGTTGAGAT GCCCGATAAG ATTAAGTCCA AAGAGATCTT 29151 TCGGGGTTGG GGTTATTCTC TGTCTTGTGA TTCTCTTTAT TCTTATACTA AGCCCCAACC CCAATAAGAG ACAGAACACT AAGAGAAATA AGAATATGAT 29201 ACGCTTCTCT GCCTAAGGCT CGCCGCCTGC TGTGTGCACA TTTGCATTTA TGCGAAGAGA CGGATTCCGA GCGGCGGACG ACACACGTGT AAACGTAAAT 29251 TTGTCAGCTT TTTAAACGCT GGGGTCGCCA CCCAAGATGA TTAGGTACAT AACAGTCGAA AAATTTGCGA CCCCAGCGGT GGGTTCTACT AATCCATGTA 29301 AATCCTAGGT TTACTCACCC TTGCGTCAGC CCACGGTACC ACCCAAAAGG .TTAGGATCA AATGAGTGGG AACGCAGTCG GGTGCCATGG TGGGTTTTCC 29351 TGGATTTTAA GGAGCCAGCC TGTAATGTTA CATTCGCAGC TGAAGCTAAT ACCTAAAATT CCTCGGTCGG ACATTACAAT GTAAGCGTCG ACTTCGATTA



29451	TCGCCACAAA AGCGGTGTTT	AACAAAATTG TTGTTTTAAC	GCAAGTATGC CGTTCATACG	TGTTTATGCT ACAAATACGA	ATTTGGCAGC TAAACCGTCG
29501	CAGGTGACAC GTCCACTGTG	TACAGAGTAT ATGTCTCATA	AATGTTACAG TTACAATGTC	TTTTCCAGGG AAAAGGTCCC	TAAAAGTCAT ATTTTCAGTA
29551			TCCATTTTAT AGGTAAAATA		
29601			AGTTGTGGCC TCAACACCGG		
29651	ACACTGGCAC TGTGACCGTG	TTTCTGCTGC AAAGACGACG	ACTGCTATGC TGACGATACG	TAATTACAGT ATTAATGTCA	GCTCGCTTTG CGAGCGAAAC
29701			TAAATACAAA ATTTATGTTT		
29751			TTACTAAGTT AATGATTCAA		
29801	TTGACGAAAT	GAGCGACGAA	GCAAAACAAA CGTTTTGTTT	AAGTTTTTCA	ATCGTAATAT
29851	TAATCTTATC	CTAAATTTGG	CCCCGGTCAT 'GGGGCCAGTA	AAGGACGAGT	TATGGTAAGG
29901	GGACTTGTTA	ACTGAGATAC	TGGGATATGC ACCCTATACG	AGGTCGCGAT	GTTGGAACTT
29951	CAGTCCGAAG	GACCTACAGT	GCATCTGACT CGTAGACTGA	AACCGGTCGT	GGACAGGGCG
30001	CCTAAACAAG	GTCAGGTTGA	ACAGCGACCC TGTCGCTGGG	TGGGATTGTC	TCTACTGGTT
30051	GTGTTGGTTG	CGCCGGCGGC	CTACCGGACT GATGGCCTGA	ATGTAGATGG	TGTTTATGTG
		ACGGAAACAG	TTATTGACCC	TATTGAACCC	GTACACCACC
		GCGAATACAA	ACATACGGAA	TAATAATACA	CCGAGTAGAC
		GCGTTTGCGC	GGGCTGGTGG	GTAGATATCA	GGGTAGTAAC
	ACGATGTGGG	TTTGTTACTA	GGAATCCATA CCTTAGGTAT	CTAACCTGCC	TGACTTTGTG
30301	ATGTTCTTTT TACAAGAAAA	CTCTTACAGT GAGAATGTCA	ATGATTAAAT TACTAATTTA	GAGACATGAT CTCTGTACTA	TCCTCGAGTT AGGAGCTCAA

Figure 26 AF

30401				TCCAGCCTTC AGGTCGGAAG	
30451				TCTGCAGCCT AGACGTCGGA	
30501	- · ·			GTCTGTGTGC CAGACACACG	
.30551				GACTATAGCT CTGATATCGA	
30601				TTTTCTGCTG AAAAGACGAC	
30651				AGCCTCAAAG TCGGAGTTTC	
30701		-		AGTTGCTACA TCAACGATGT	
30751				CATCTCTGTT GTAGAGACAA	
30801			_	CCTACCTTGA GGATGGAACT	
30851				TTCCCCGCGC AAGGGGCGCG	
30901				TGTCCCAGCC ACAGGGTCGG	
30951			- ·	GCTACTTTAA CGATGAAATT	
31001				GGACGGAATT CCTGCCTTAA	
31051				CCGAGCAACA GGCTCGTTGT	
31101	CAAGAGCTCC GTTCTCGAGG			CAGTGCAAAA GTCACGTTTT	
	TTGTCTCGTA AACAGAGCAT				
31201	ACCGCCTTAG TGGCGGAATC			GTCAGAAATT CAGTCTTTAA	
31251	GTGGGAGAAA CACCCTCTTT			CACTCGGTAG GTGAGCCATC	

Figure 26 A6 87/144

31351		 		ATAAAAAAA TATTTTTTTT
31401			TAGCAAATTT ATCGTTTAAA	CTGTCCAGTT GACAGGTCAA
31451		 	AGCTCTGGTA TCGAGACCAT	
31501	•	 	AATGGAATGT TTACCTTACA	
31551			CATGTTGTTG GTACAACAAC	
31601			CCGTGTATCC GGCACATAGG	
31651			ACTCCTCCCT TGAGGAGGGA	
31701	•. • • • • • • •	 	ACTCTCTTTG TGAGAGAAAC	
31751			CGCTCAAAAT GCGAGTTTTA	
31801			TCCCAAAATG AGGGTTTTAC	
31851			CATAAACCTG GTATTTGGAC	
31901			CTGTGGCTGC GACACCGACG	
31951	•		CAATCACAGG GTTAGTGTCC	
	CGTGCACGAC GCACGTGCTG			CTCACAGTGT GAGTGTCACA
32051	CAGAAGGAAA GTCTTCCTTT		GCCCCCTCAC CGGGGGAGTG	
32101	AGCAGTACCC TCGTCATGGG		CCTCTAACTA GGAGATTGAT	
32151	TAGCTTGGGC ATCGAACCCG		TTATACACAA AATATGTGTT	
32201	TAGGACTAAA ATCCTGATTT			

Figure 26 AH

32301	AACTAAAGTT TTGATTTCAA	ACTGGAGCCT TGACCTCGGA		
32351	•	AGGAGGACTA TCCTCCTGAT	 	ACGCCTTATA TGCGGAATAT
32401	•	GTTATCCGTT CAATAGGCAA		ATCTAAGACT TAGATTCTGA
32451	AGGACAGGGC TCCTGTCCCG	CCTCTTTTTA GGAGAAAAAT	 	•
32501		CCTTTACTTG GGAAATGAAC		
32551	•	TAAGCACTGC ATTCGTGACG		
32601		GCAGGAGATG CGTCCTCTAC		
32651		CCTCAAAACA GGAGTTTTGT		
32701		TGGTTCCTAA ACCAAGGATT	 	
32751		ACAGTAGGAA TGTCATCCTT		
32801		TCCATCTCCT AGGTAGAGGA		
32851		TGGTCTTAAC ACCAGAATTG		
32901		GCTGTTAAAG CGACAATTTC		
32951		TCTTATTATA AGAATAATAT		•
33001	AATTCCTTCC TTAAGGAAGG	TGGACCCAGA ACCTGGGTCT		
33051	TGAAGGCACA ACTTCCGTGT	GCCTATACAA CGGATATGTT		
33101	CTTATCCAAA GAATAGGTTT	ATCTCACGGT TAGAGTGCCA		
33151	GTTTACTTAA CAAATGAATT	ACGGAGACAA TGCCTCTGTT		

Figure 26 AI 89/144

33251	CATTTTCATG	GGACTGGTCT	GGCCACAACT	<b>ACATTAATGA</b>	AATATTTGCC
55252	CTAAAACTAC	CCTGACCAGA	CCGGTGTTGA	TGTAATTACT	TTATAAACGG
	GIMMAGIAC	cc i dilicciidi.			
			ATACATTGCC	CAACAATAAA	GAATCGTTTG
33301	ACATCCTCTT	ACACTITITC	ATACATIGCC	CURCULATION	CTTACCAAAC
	TGTAGGAGAA	TGTGAAAAAG	TATGTAACGG	GTTCTTATTT	CITAGCAAAC
33351	TGTTATGTTT	CAACGTGTTT	ATTTTTCAAT	TGCAGAAAAT	TTCAAGTCAT
	ACAATACAAA	GTTGCACAAA	TAAAAAGTTA	ACGTCTTTTA	<b>AAGTTCAGTA</b>
	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,				
	TTTTCATTCA	OD1 OD1 01 00	COCACCACCA	СУФУССФФУТ	ACAGATCACC
33401	TTTTCATICA	GTAGTATAGC	CCCACCACCA	CMINGCIAN	TOTOTAGTGG
	AAAAGTAAGT	CATCATATCG	GGGTGGTGGT	GIMICGAAIA	1010170100
33451	GTACCTTAAT	CAAACTCACA	GAACCCTAGT	ATTCAACCTG	CCACCTCCCT
	CATGGAATTA	GTTTGAGTGT	CTTGGGATCA	TAAGTTGGAC	GGTGGAGGGA
22501	CCCNACACAC	AGAGTACACA	GTCCTTTCTC	CCCGGCTGGC	CTTAAAAAGC
33501			CAGGAAAGAG		GAATTTTTCG
	GGGTTGTGTG	TCTCATGIGI	CAGGAMGAG	000000.000	<b>0.5</b> (1)
				ocmcomp.m.m.m.	TCCACACGGT
33551	ATCATATCAT	GGGTAACAGA	CATATTCTTA	001011111	
	TAGTATAGTA	CCCATTGTCT	GTATAAGAAT	CCACAATATA	AGGTGTGCCA
33601	TTCCTGTCGA	GCCAAACGCT	CATCAGTGAT	ATTAATAAAC	TCCCCGGGCA
33001	AAGGACAGCT	CGGTTTGCGA	GTAGTCACTA	TAATTATTTG	AGGGGCCCGT
	Anddhenoe:				
	0000100011		CTGTCCAGCT	CCTGAGCCAC	AGGCTGCTGT
33651	GCTCACTTAA	GITCAIGICG	GACAGGTCGA	CCACTCGGTG	TCCGACGACA
	CGAGTGAATT	CAAGTACAGC	GACAGGICGA	CGACICGGIG	10001100
					> 0000m>C>m
33701	CCAACTTGCG	GTTGCTTAAC	GGGCGGCGAA	GGAGAAGTCC	ACGCCTACAT
	GGTTGAACGC	CAACGAATTG	CCCGCCGCTT	CCTCTTCAGG	TGCGGATGTA
				•	
33751	GGGGGTAGAG	TCATAATCGT	GCATCAGGAT	AGGGCGGTGG	TGCTGCAGCA
33,31	CCCCCATCTC	ACTATTACCA	CGTAGTCCTA	TCCCGCCACC	ACGACGTCGT
	CCCCATCTC	AGIAIIAGEA			
			CĠCCGCCGCT	CCCTCCTCCA	CCAATACAAC
33801	GCGCGCGAAT	AAACTGCTGC	CGCCGCCGC1	CCCTCCTCCA	CCTTATCTTC
	CGCGCGCTTA	TTTGACGACG	GCGGCGGCGA	GGCAGGACGI	CCTIATGITG
		·			
33851	ATGGCAGTGG	TCTCCTCAGC	GATGATTCGC	ACCGCCCGCA	GCATAAGGCG
	TACCGTCACC	AGAGGAGTCG	CTACTAAGCG	TGGCGGGCGT	CGTATTCCGC
		•			
22001	CCTTGTCCTC	רככככר אר אכר	AGCGCACCCT	GATCTCACTT	AAATCAGCAC
33901	0011010010	CCCCCTCTCC	TOCCOTTOGGA	CTAGAGTGAA	TTTAGTCGTG
	GGAACAGGAG	GCCCGIGICG	1000010001		• • • • • • • • • • • • • • • • • • • •
				maxxxxxx	ACACTCCAAC
33951	AGTAACTGCA	GCACAGCACC	ACAATATIGT	TUAAAATUUU	ACAGTGCAAG
	TCATTGACGT	CGTGTCGTGG	TGTTATAACA	AGTTTTAGGG	TGTCACGTTC
34001	GCGCTGTATC	CAAAGCTCAT	GGCGGGGACC	ACAGAACCCA	CGTGGCCATC
24001	CGCGACATAC	GTTTCGAGTA	CCGCCCCTGG	TGTCTTGGGT	GCACCGGTAG
	COCONCAING				
		0001000101	MTS S CTCCCC	* አርርርር ተስርልጥል	AACACGCTGG
34051	ATACCACAAG	CGCAGGTAGA	1199010000	MCCCC CONTR	TTGTGCGACC
	TATGGTGTTC	GCGTCCATCT	AATTCACCGC	10000AG1A1	TTGTGCGACC
			•		
34101	ACATAAACAT	TACCTCTTTT	GGCATGTTGT	AATTCACCAC	CTCCCGGTAC
	TGTATTTGTA	ATGGAGAAAA	CCGTACAACA	TTAAGTGGTG	GAGGGCCATG

Figure 26 AJ

				,	
34201	GCTGGCCAAA	ACCTGCCCGC	CGGCTATACA	CTGCAGGGAA	CCGGGACTGG
•	CGACCGGTTT	TGGACGGGCG	GCCGATATGT	GACGTCCCTT	GGCCCTGACC
34251	AACAATGACA	GTGGAGAGCC	CAGGACTCGT	AACCATGGAT	CATCATGCTC
	TTGTTACTGT	CACCTCTCGG	GTCCTGAGCA	TTGGTACCTA	GTAGTACGAG
34301	GTCATGATAT	CAATGTTGGC	ACAACACAGG	CACACGTGCA	TACACTTCCT
	CAGTACTATA	GTTACAACCG	TGTTGTGTCC	GTGTGCACGT	ATGTGAAGGA
34351	CAGGATTACA	AGCTCCTCCC	GCGTTAGAAC	CATATCCCAG	GGAACAACCC
	GTCCTAATGT	TCGAGGAGGG	CGCAATCTTG	GTATAGGGTC	CCTTGTTGGG
34401	ATTCCTGAAT	CAGCGTAAAT	CCCACACTGC	AGGGAAGACC	TCGCACGTAA
	TAAGGACTTA	GTCGCATTTA	GGGTGTGACG	TCCCTTCTGG	AGCGTGCATT
34451	CTCACGTTGT	GCATTGTCAA	AGTGTTACAT	TCGGGCAGCA	GCGGATGATC
	GAGTGCAACA	CGTAACAGTT	TCACAATGTA	AGCCCGTCGT	CGCCTACTAG
34501				AAAAGGAGGT	
	GAGGTCATAC	CATCGCGCCC	AAAGACAGAG	TTTTCCTCCA	TCTGCTAGGG
34551				ATCGTGTTGG	
	ATGACATGCC	TCACGCGGCT	CTGTTGGCTC	TAGCACAACC	AGCATCACAG
				•	
34601				TTTCCTGAAG	
	. TACGGTTTAC	CTTGCGGCCT	GCATCAGTAT	AAAGGACTTC	GTTTTGGTCC
34651				GGTCTCGCCG	
	ACGCCCGCAC	TGTTTGTCTA	GACGCAGAGG	CCAGAGCGGC	GAATCTAGCG
24703	mamamama ar	1 CMMCM1 CM1	mamaca a conco	CTCAAAGCAT	001000000
34701				GAGTTTCGTA	
	AGACACATCA	ICAACAICAI	AIAGGIGAGA	GAGITICGIA	GGTCCGCGG
34751	CCMCCCMMCC	CCTTCTATCT	3 3 3 CTCCTTC	ATGCGCCGCT	CCCCTCATAA
34177	•		•	TACGCGCCGCT	
	GGACCGAAGC	CCAAGATACA	1110AGGAAG	INCOCOGCON	COGGACIAII
34801	CATCCACCAC	CCCACAATAA	GCCACACCCA	GCCAACCTAC	<b>እ</b> ሮ <b>እ</b> ምምርርምምር
34001				CGGTTGGATG	
	01A0010010	oco.c	0001010001	COOTTOONIG	TOTALGENIO
34851	TGCGAGTCAC	ACACGGGAGG	AGCGGGAAGA	GCTGGAAGAA	CCATGTTTTT
0.05.				CGACCTTCTT	
34901	TTTTTTATTC	CAAAAGATTA	TCCAAAACCT	CAAAATGAAG	ATCTATTAAG
				GTTTTACTTC	
34951	TGAACGCGCT	CCCCTCCGGT	GGCGTGGTCA	AACTCTACAG	CCAAAGAACA
	ACTTGCGCGA	GGGGAGGCCA	CCGCACCAGT	TTGAGATGTC	GGTTTCTTGT
				_	
35001	GATAATGGCA	TTTGTAAGAT	GTTGCACAAT	GGCTTCCAAA	AGGCAAACGG
				CCGAAGGTTT	
35051	CCCTCACGTC	CAAGTGGACG	TAAAGGCTAA	ACCCTTCAGG	GTGAATCTCC
	GGGAGTGCAG	GTTCACCTGC	ATTTCCGATT	TGGGAAGTCC	CACTTAGAGG

Figure 26 AK

PCT/US01/28861 WO 02/022080

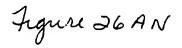
				•	
35151	CCACCTTCTC	AATATATCTC	TAAGCAAATC	CCGAATATTA	AGTCCGGCCA
33232	GGTGGAAGAG	TTATATAGAG	ATTCGTTTAG	GGCTTATAAT	TCAGGCCGGT
	00.00.0				
35201	ттставават	CTGCTCCAGA	GCGCCCTCCA	CCTTCAGCCT	CAAGCAGCGA
33202	ΑΤΤΤΤΤΑ	GACGAGGTCT	CGCGGGAGGT	GGAAGTCGGA	GTTCGTCGCT
	Michie	0			
35251	ATCATGATTG	CAAAAATTCA	GGTTCCTCAC	AGACCTGTAT	AAGATTCAAA
JJ2J2	TACTACTAAC	GTTTTTAAGT	CCAAGGAGTG	TCTGGACATA	TTCTAAGTTT
	indincinc	0			
35301	ACCCCAACAT	TAACAAAAAT	ACCGCGATCC	CGTAGGTCCC	TTCGCAGGGC
33301	TOCCOMMENT	ATTGTTTTTA	TGGCGCTAGG	GCATCCAGGG	AAGCGTCCCG
	icacciioin	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,			
35351	CACCTGAACA	TAATCGTGCA	GGTCTGCACG	GACCAGCGCG	GCCACTTCCC
33331	CTCCACTTCT	ATTAGCACGT	CCAGACGTGC	CTGGTCGCGC	CGGTGAAGGG
	GICGACIIGI	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,			
35401	CCCCAGGAAC	CATGACAAAA	GAACCCACAC	TGATTATGAC	ACGCATACTC
22401	CCCCACCATC	GTACTGTTTT	CTTGGGTGTG	ACTAATACTG	TGCGTATGAG
	GCGGTCCTTG	01/10101111			
35451	CCACCTATCC	TAACCAGCGT	AGCCCCGATG	TAAGCTTGTT	GCATGGGCGG
22421	CCTCGATACG	ATTGGTCGCA	TCGGGGCTAC	ATTCGAACAA	CGTACCCGCC
	CCTCGATACG				
35501	ССУДУДУУ	TGCAAGGTGC	TGCTCAAAAA	ATCAGGCAAA	GCCTCGCGCA
35501	CCTATATTT	ACGTTCCACG	ACGAGTTTTT	TAGTCCGTTT	CGGAGCGCGT
	GCIMIMILI				
35551	DEADAGAAG	CACATCGTAG	TCATGCTCAT	GCAGATAAAG	GCAGGTAAGC
33331	**************************************	GTGTAGCATC	AGTACGAGTA	CGTCTATTTC	CGTCCATTCG
	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	010111001110			
35601	TCCGGAACCA	CCACAGAAAA	AGACACCATT	TTTCTCTCAA	ACATGTCTGC
33001	ACCCCTTGCT	GGTGTCTTTT	TCTGTGGTAA	AAAGAGAGTT	TGTACAGACG
	AGGCC110G1	00.0.0			
35651	GGGTTTCTGC	ATAAACACAA	AATAAAATAA	CAAAAAAACA	TTTAAACATT
33031	CCCAAAGACG	TATTTGTGTT	TTATTTTATT	GTTTTTTTGT	AAATTTGTAA
	CCC/111.000				
35701	AGAAGCCTGT	CTTACAACAG	GAAAAACAAC	CCTTATAAGC	ATAAGACGGA
33.01	TCTTCGGACA	GAATGTTGTC	CTTTTTGTTG	GGAATATTCG	TATTCTGCCT
	1011100				
35751	CTACGGCCAT	GCCGGCGTGA	CCGTAAAAA	ACTGGTCACC	GTGATTAAAA
33,32	GATGCCGGTA	CGGCCGCACT	GGCATTTTTT	TGACCAGTGG	CACTAATTTT
	<b>G.</b>				
35801	AGCACCACCG	ACAGCTCCTC	GGTCATGTCC	GGAGTCATAA	TGTAAGACTC
33001	TOTTGTGGC	TGTCGAGGAG	CCAGTACAGG	CCTCAGTATT	ACATTCTGAG
35851	GGTAAACACA	TCAGGTTGAT	TCACATCGGT	CAGTGCTAAA	AAGCGACCGA
33031	CCATTTGTGT	AGTCCAACTA	AGTGTAGCCA	GTCACGATTT	TTCGCTGGCT
35901	AATAGCCCGG	GGGAATACAT	ACCCGCAGGC	GTAGAGACAA	CATTACAGCC
22242	TTATCGGGCC	CCCTTATGTA	TGGGCGTCCG	CATCTCTGTT	GTAATGTCGG
				•	
35051	CCCATAGGAG	GTATAACAAA	ATTAATAGGA	GAGAAAAACA	CATAAACACC
٠ <i>٠ ٠ ٠ ٠</i>	CCCTATCCTC	CATATTGTTT	TAATTATCCT	CTCTTTTTGT	GTATTTGTGG
	200				
36001	TGAAAAACCC	TCCTGCCTAG	GCAAAATAGC	ACCCTCCCGC	TCCAGAACAA
20001	ACTTTTTCCC	AGGACGGATC	CGTTTTATCG	TGGGAGGGCG	AGGTCTTGTT

Figure 26 AL

36101	AAAGAAAACC			ACACGGCACC TGTGCCGTGG	
36151				GCGAGTATAT CGCTCATATA	
36201				AACACCCAGA TTGTGGGTCT	
36251				AACCCACAAC TTGGGTGTTG	
36301			-	TTCCCATTTT AAGGGTAAAA	
36351				CTAAAACCTA GATTTTGGAT	
36401				ACTCCACCC TGAGGTGGGG	
					PacI
36451	TATTGGCTTC	AATCCAAAAT	AAGGTATATT	ATTGATGATG	TTAATTAAGA
				TAACTACTAC	
36501				CTTCCCCATT GAAGGGGTAA	
36551				TGCAGGCCAT ACGTCCGGTA	
36601				CAAGGCCAGC GTTCCGGTCG	
36651				TTTCCATAGG AAAGGTATCC	
36701				GTCAGAGGTG CAGTCTCCAC	
36751	ACAGGACTAT TGTCCTGATA			CCTGGAAGCT GGACCTTCGA	
36801	CTCTCCTGTT GAGAGGACAA			ATACCTGTCC TATGGACAGG	
36851	CTTCGGGAAG GAAGCCCTTC			CACGCTGTAG GTGCGACATC	
36901	TCGGTGTAGG AGCCACATCC			TGTGTGCACG ACACACGTGC	

Figure 26 AM

37001	CGGTAAGACA	CGACTTATCG	CCACTGGCAG	CAGCCACTGG	TAACAGGATT
	GCCATTCTGT	GCTGAATAGC	GGTGACCGTC	GTCGGTGACC	ATTGTCCTAA
37051		GGTATGTAGG CCATACATCC			
37101	TAACTACGGC	TACACTAGAA	GGACAGTATT	TGGTATCTGC	GCTCTGCTGA
	ATTGATGCCG	ATGTGATCTT	CCTGTCATAA	ACCATAGACG	CGAGACGACT
37151	AGCCAGTTAC	CTTCGGAAAA	AGAGTTGGTA	GCTCTTGATC	CGGCAAACAA
	TCGGTCAATG	GAAGCCTTTT	TCTCAACCAT	CGAGAACTAG	GCCGTTTGTT
37201	ACCACCGCTG	GTAGCGGTGG	TTTTTTTGTT	TGCAAGCAGC	AGATTACGCG
	TGGTGGCGAC	CATCGCCACC	AAAAAAACAA	ACGTTCGTCG	TCTAATGCGC
37251	CAGAAAAAA	GGATCTCAAG	AAGATCCTTT	GATCTTTTCT	ACGGGGTCTG
	GTCTTTTTT	CCTAGAGTTC	TTCTAGGAAA	CTAGAAAAGA	TGCCCCAGAC
37301	ACGCTCAGTG	GAACGAAAAC	TCACGTTAAG	GGATTTTGGT	CATGAGATTA
	TGCGAGTCAC	CTTGCTTTTG	AGTGCAATTC	CCTAAAACCA	GTACTCTAAT
37351	TCAAAAAGGA	TCTTCACCTA	GATCCTTTTA	AATCAATCTA	AAGTATATAT
	AGTTTTTCCT	AGAAGTGGAT	CTAGGAAAAT	TTAGTTAGAT	TTCATATATA
37401	GAGTAAACTT	GGTCTGACAG	TTACCAATGC	TTAATCAGTG	AGGCACCTAT
	CTCATTTGAA	CCAGACTGTC	AATGGTTACG	AATTAGTCAC	TCCGTGGATA
37451	CTCAGCGATC	TGTCTATTTC	GTTCATCCAT	AGTTGCCTGA	CTCCCCGTCG
	GAGTCGCTAG	ACAGATAAAG	CAAGTAGGTA	TCAACGGACT	GAGGGGCAGC
37501	ACATCTATTG	TACGATACGG ATGCTATGCC	CTCCCGAATG	GTAGACCGGG	GTCACGACGT
37551	TACTATGGCG	GAGACCCACG CTCTGGGTGC	GAGTGGCCGA	GGTCTAAATA	GTCGTTATTT
37601	CCAGCCAGCC	GGAAGGGCCG	AGCGCAGAAG	TGGTCCTGCA	ACTTTATCCG
	GGTCGGTCGG	CCTTCCCGGC	TCGCGTCTTC	ACCAGGACGT	TGAAATAGGC
37651	GGAGGTAGGT	GTCTATTAAT CAGATAATTA	ACAACGGCCC	TTCGATCTCA	TTCATCAAGC
	GGTCAATTAT	CAAACGCGTT	GCAACAACGG	TAACGATGTC	GCATCGTGGT CGTAGCACCA
	CAGTGCGAGC	AGCAAACCAT	ACCGAAGTAA	GTCGAGGCCA	TCCCAACGAT AGGGTTGCTA
37801	CAAGGCGAGT	TACATGATCC	CCCATGTTGT	GCAAAAAAGC	GGTTAGCTCC
	GTTCCGCTCA	ATGTACTAGG	GGGTACAACA	CGTTTTTTCG	CCAATCGAGG
37851	TTCGGTCCTC	CGATCGTTGT	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT
	AAGCCAGGAG	GCTAGCAACA	GTCTTCATTC	AACCGGCGTC	ACAATAGTGA



37951	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	CCAAGTCATT	CTGAGAATAG
	CTACGAAAAG	ACACTGACCA	CTCATGAGTT	GGTTCAGTAA	GACTCTTATC
38001	TGTATGCGGC				
			GAGAACGGGC		
38051			TAAAAGTGCT		
			ATTTTCACGA		
38101			ATCTTACCGC		
			TAGAATGGCG		
38151	TAACCCACTC				
			GACTAGAAGT		
38201			CAGGAAGGCA		
			GTCCTTCCGT		
38251	TAAGGGCGAC				
			ACTTATGAGT		
38301			TTATTGTCTC		
			AATAACAGAG		
38351			AAATAGGGGT		
			TTTATCCCCA		
38401	AAGTGCCACC				
		·	CTTTGGTAAT		
38451	AAAAATAGGC				
	TTTTTATCCG	CATAGTGCTC	CGGGAAAGCA	GAAGTTCTTA	ACCTAGGCTT
		PacI			
			/850 TD	221	
	TTCTTAATTT				
	AAGAATTAAA	GAATTAATT	(PEG ID NO:	: 331	

Figure 26 AO

1	CATCATCAAT	AATATACCTT	ATTTTGGATT	GAAGCCAATA CTTCGGTTAT	TGATAATGAG
51	GGGGTGGAGT	TTGTGACGTG	GCGCGGGGGCG	TGGGAACGGG ACCCTTGCCC	CCCCCACTCC
101	TAGTAGTGTG	GCGGAAGTGT	GATGTTGCAA	GTGTGGCGGA	ACACATGTAA
				CACACCGCCT	
151	GCGACGGATG	TGGCAAAAGT	GACGTTTTTG	GTGTGCGCCG	GTGTACACAG
				CACACGCGGC	
201	GAAGTGACAA	TTTTCGCGCG	GTTTTAGGCG	GATGTTGTAG	TAAATTTGGG
	CTTCACTGTT	AAAAGCGCGC	CAAAATCCGC	CTACAACATC	ATTTAAACCC
251	CGTAACCGAG	TAAGATTTGG	CCATTTTCGC	GGGAAAACTG	AATAAGAGGA
	GCATTGGCTC	ATTCTAAACC	GGTAAAAGCG	CCCTTTTGAC	TTATTCTCCT
301	AGTGAAATCT	GAATAATTTT	GTGTTACTCA	TAGCGCGTAA	TATTTGTCTA
	TCACTTTAGA	СТТАТТАЛАЛ	CACAATGAGT	ATCGCGCATT	ATAAACAGAT
351	GGGCCGCGG	GACTTTGACC	GTTTACGTGG	AGACTCGCCC	AGGTGTTTTT
	CCCGGCGCCC	CTGAAACTGG	CAAATGCACC	TCTGAGCGGG	TCCACAAAAA
401	CTCAGGTGTT	TTCCGCGTTC	CGGGTCAAAG	TTGGCGTTTT	ATTATTATAG
	GAGTCCACAA	AAGGCGCAAG	GCCCAGTTTC	AACCGCAAAA	TAATAATATC
451	GCGGCCGCGA	TCCATTGCAT	ACGTTGTATC	CATATCATAA	TATGTACATT
				GTATAGTATT	
501	TATATTGGCT	CATGTCCAAC	ATTACCGCCA	TGTTGACATT	GATTATTGAC
	•			ACAACTGTAA	
551	TAGTTATTAA	TAGTAATCAA	TTACGGGGTC	ATTAGTTCAT	AGCCCATATA
				TAATCAAGTA	
601	TGGAGTTCCG	CGTTACATAA	CTTACGGTAA	ATGGCCCGCC	TGGCTGACCG
				TACCGGGCGG	
651	CCCAACGACC	CCCGCCCATT	GACGTCAATA	ATGACGTATG	TTCCCATAGT
				TACTGCATAC	
701	AACGCCAATA	GGGACTTTCC	ATTGACGTCA	ATGGGTGGAG	TATTTACGGT
	TTGCGGTTAT	CCCTGAAAGG	TAACTGCAGT	TACCCACCTC	ATAAATGCCA
751	AAACTGCCCA	CTTGGCAGTA	CATCAAGTGT	ATCATATGCC	AAGTACGCCC
	TTTGACGGGT	GAACCGTCAT	GTAGTTCACA	TAGTATACGG	TTCATGCGGG
801	CCTATTGACG	TCAATGACGG	TAAATGGCCC	GCCTGGCATT	ATGCCCAGTA
	GGATAACTGC	AGTTACTGCC	ATTTACCGGG	CGGACCGTAA	TACGGGTCAT

Figure 27A

901					TGGGCGTGGA
	AGCGATAATG	GTACCACTAC	GCCAAAACCG	F TCATGTAGTT	ACCCGCACCT
951					TTGACGTCAA
	ATCGCCAAAC	TGAGTGCCCC	TAAAGGTTCA	GAGGTGGGG1	AACTGCAGTT
1001					AAATGTCGTA
	ACCCTCAAAC	AAAACCGTGG	TTTTAGTTGC	CCTGAAAGGT	TTTACAGCAT
1051					ACGGTGGGAG
	TGTTGAGGCG	GGGTAACTGC	GTTTACCCGC	CATCCGCACA	TGCCACCCTC
1101					CCTGGAGACG
		CGTCTCGAGC			
1151		TGTTTTGACC			
		ACAAAACTGG			
1201					TGCCAAGAGT
		CCTTGCCACG			
1251		ACCATGGCCG			
					CACGGGCCGA
1301		GAGGGAGAGG			
		CTCCCTCTCC			
1351		CCGAGCCCGC			
		GGCTCGGGCG			
1401		CACGGCGCCA			
		GTGCCGCGGT			
1451		CTGGCTGGAG			
		GACCGACCTC			
1501		AGGTGCCCCT			
		TCCACGGGGA			
1551		TTCCTGAAGG			
		AAGGACTTCC			
1601	• • • • • • • • • • • • • • • • • • • •	GCAGGACATC			
		CGTCCTGTAG			
1651		ACTGGCAGAA			
		TGACCGTCTT			
1701		GGCTGGTGCT			
	GGACTGGAAG	CCGACCACGA	AGTTCGACCA	CGGGCACCTC	GGGCTCTTCC
		CAACGAGGGC			
	ACCTCCTCCG	GTTGCTCCCG	CTCTTGTTGA	CGCGGCGGGT	GGGGTACAGG

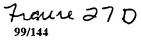
Figure 27B

PCT/US01/28861 WO 02/022080

1851	CTCCAAGCTG	GCCTTCCACC	ACGTGGCCAG	GGAGCTGCAC	CCCGAGTACT
	GAGGTTCGAC	CGGAAGGTGG	TGCACCGGTC	CCTCGACGTG	GGGCTCATGA
1901		CTAAAGCCCG GATTTCGGGC			
1951		TTTGCCCCTC AAACGGGGAG			
2001	CACTCCCACT	GTCCTTTCCT	AATAAAATGA	GGAAATTGCA	TCGCATTGTC
	GTGAGGGTGA	CAGGAAAGGA	TTATTTTACT	CCTTTAACGT	AGCGTAACAG
2051	TGAGTAGGTG	TCATTCTATT	CTGGGGGGTG	GGGTGGGGCA	GGACAGCAAG
	ACTCATCCAC	AGTAAGATAA	GACCCCCCAC	CCCACCCCGT	CCTGTCGTTC
2101		GGGAAGACAA CCCTTCTGTT			
2151		CGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC			
2201	GGGAAAGAAT	ATATAAGGTG	GGGGTCTTAT	GTAGTTTTGT	ATCTGTTTTG
	CCCTTTCTTA	TATATTCCAC	CCCCAGAATA	CATCAAAACA	TAGACAAAAC
2251	CAGCAGCCGC	CGCCGCCATG	AGCACCAACT	CGTTTGATGG	AAGCATTGTG
	GTCGTCGGCG	GCGGCGGTAC	TCGTGGTTGA	GCAAACTACC	TTCGTAACAC
2301	AGCTCATATT	TGACAACGCG	CATGCCCCCA	TGGGCCGGGG	TGCGTCAGAA
	TCGAGTATAA	ACTGTTGCGC	GTACGGGGGT	ACCCGGCCCC	ACGCAGTCTT
2351		TCCAGCATTG AGGTCGTAAC			
2401	CTACCTTGAC	CTACGAGACC	GTGTCTGGAA	CGCCGTTGGA	GACTGCAGCC
	GATGGAACTG	GATGCTCTGG	CACAGACCTT	GCGGCAACCT	CTGACGTCGG
2451	TCCGCCGCCG	CTTCAGCCGC	TGCAGCCACC	GCCCGCGGGA	TTGTGACTGA
	AGGCGGCGGC	GAAGTCGGCG	ACGTCGGTGG	CGGGCGCCCT	AACACTGACT
2501	CTTTGCTTTC	CTGAGCCCGC	TTGCAAACAĠ	TGCAGCTTCC	CGTTCATCCG
	GAAACGAAAG	GACTCGGGCG	AACGTTTGTC	ACGTCGAAGG	GCAAGTAGGC
2551	CCCGCGATGA GGGCGCTACT	CAAGTTGACG GTTCAACTGC	GCTCTTTTGG CGAGAAAACC	CACAATTGGA GTGTTAACCT	TTCTTTGACC AAGAAACTGG
2601	CGGGAACTTA	ATGTCGTTTC	TCAGCAGCTG	TTGGATCTGC	GCCAGCAGGT
	GCCCTTGAAT	TACAGCAAAG	AGTCGTCGAC	AACCTAGACG	CGGTCGTCCA
2651	TTCTGCCCTG	AAGGCTTCCT	CCCCTCCCAA	TGCGGTTTAA	AACATAAATA
	AAGACGGGAC	TTCCGAAGGA	GGGGAGGGTT	ACGCCAAATT	TTGTATTTAT
2701	AAAAACCAGA	CTCTGTTTGG	ATTTGGATCA	AGCAAGTGTC	TTGCTGTCTT
	TTTTTGGTCT	GAGACAAACC	TAAACCTAGT	TCGTTCACAG	AACGACAGAA

Figure 27 C

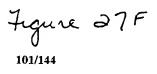
2751	TATTTAGGGG	TTTTGCGCGC	GCGGTAGGCC	CGGGACCAGC	GGTCTCGGT
	ATAAATCCCC	AAAACGCGCG	CGCCATCCGG	GCCCTGGTCG	CCAGAGCCAC
2801	GTTGAGGGTC	CTGTGTATTT	TTTCCAGGAC	GTGGTAAAGG	TGACTCTGGA
	CAACTCCCAG	GACACATAAA	AAAGGTCCTG	CACCATTTCC	ACTGAGACCT
2851	-		AGCCCGTCTC		
	ACAAGTCTAT	GTACCCGTAT	TCGGGCAGAG	ACCCCACCTC	CATCGTGGTG
2901	TGCAGAGCTT	CATGCTGCGG	GGTGGTGTTG	TAGATGATCC	AGTCGTAGCA
	ACGTCTCGAA	GTACGACGCC	CCACCACAAC	ATCTACTAGG	TCAGCATCGT
2951	GGAGCGCTGG	GCGTGGTGCC	TAAAAATGTC	TTTCAGTAGC	AAGCTGATTG
	CCTCGCGACC	CGCACCACGG	ATTTTTACAG	AAAGTCATCG	TTCGACTAAC
3001	CCAGGGGCAG	GCCCTTGGTG	TAAGTGTTTA	CAAAGCGGTT	AAGCTGGGAT
			ATTCACAAAT		
3051			GAGATGCATC		
	CCCACGTATG	CACCCCTATA	CTCTACGTAG	AACCTGACAT	AAAAATCCAA
3101	GGCTATGTTC	CCAGCCATAT	CCCTCCGGGG	ATTCATGTTG	TGCAGAACCA
	CCGATACAAG	GGTCGGTATA	GGGAGGCCCC	TAAGTACAAC	ACGTCTTGGT
3151	CCAGCACAGT	GTATCCGGTG	CACTTGGGAA	ATTTGTCATG	TAGCTTAGAA
	GGTCGTGTCA	CATAGGCCAC	GTGAACCCTT	TAAACAGTAC	ATCGAATCTT
3201	GGAAATGCGT	GGAAGAACTT	GGAGACGCCC	TTGTGACCTC	CAAGATTTTC
	CCTTTACGCA	CCTTCTTGAA	CCTCTGCGGG	AACACTGGAG	GTTCTAAAAG
3251	CATGCATTCG	TCCATAATGA	TGGCAATGGG	CCCACGGGCG	GCGGCCTGGG
	GTACGTAAGC	AGGTATTACT	ACCGTTACCC	GGGTGCCCGC	CGCCGGACCC
3301	CGAAGATATT	TCTGGGATCA	CTAACGTCAT	AGTTGTGTTC	CAGGATGAGA
	GCTTCTATAA	AGACCCTAGT	GATTGCAGTA	TCAACACAAG	GTCCTACTCT
3351	TCGTCATAGG	CCATTTTTAC	AAAGCGCGGG	CGGAGGGTGC	CAGACTGCGG
	AGCAGTATCC	GGTAAAAATG	TTTCGCGCCC	GCCTCCCACG	GTCTGACGCC
3401	TATAATGGTT	CCATCCGGCC	CAGGGGCGTA	GTTACCCTCA	CAGATTTGCA
	ATATTACCAA	GGTAGGCCGG	GTCCCCGCAT	CAATGGGAGT	GTCTAAACGT
3451	TTTCCCACGC	TTTGAGTTCA	GATGGGGGGA	TCATGTCTAC	CTGCGGGGCG
	AAAGGGTGCG	AAACTCAAGT	CTACCCCCCT	AGTACAGATG	GACGCCCCGC
3501	ATGAAGAAAA	CGGTTTCCGG	GGTAGGGGAG	ATCAGCTGGG	AAGAAAGCAG
	TACTTCTTTT	GCCAAAGGCC	CCATCCCCTC	TAGTCGACCC	TTCTTTCGTC
3551	GTTCCTGAGC				
	CAAGGACTCG	TCGACGCTGA	ATGGCGTCGG	CCACCCGGGC	ATTTAGTGTG
3601	CTATTACCGG				
	GATAATGGCC	GACGTTGACC	ATCAATTCTC	TCGACGTCGA	CGGCAGTAGG
3651	CTGAGCAGGG	GGGCCACTTC	GTTAAGCATG	TCCCTGACTC	GCATGTTTTC
	GACTCGTCCC				



3701			GGCGCTCGCC CCGCGAGCGG		
3751			AACGGTTTGA TTGCCAAACT		
3801			CAGTTCCAGG GTCAAGGTCC		
3851	CTGCTCTACG GACGAGATGC	GCATCTCGAT CGTAGAGCTA	CCAGCATATC GGTCGTATAG	TCCTCGTTTC AGGAGCAAAG	GCGGGTTGGG CGCCCAACCC
3901			GTAGTCGGTG CATCAGCCAC		
3951	AGTACAGAAA	GGTGCCCGCG	AGGGTCCTCG TCCCAGGAGC	AGTCGCATCA	GACCCAGTGC
4001	CACTTCCCCA	CGCGAGGCCC	CTGCGCGCTG GACGCGCGAC	CGGTCCCACG	CGAACTCCGA
4051	CCAGGACGAC	CACGACTTCG	GCTGCCGGTC CGACGGCCAG	AAGCGGGACG	CGCAGCCGGT
4101	CCATCGTAAA	CTGGTACCAC	TCATAGTCCA AGTATCAGGT	CGGGGAGGCG	CCGCACCGGG
4151	AACCGCGCGT	CGAACGGGAA	GGAGGAGGCG CCTCCTCCGC	GGCGTGCTCC	CCGTCACGTC
4201	TGAAAACTCC	CGCATCTCGA	TGGGCGCGAG ACCCGCGCTC	TTTATGGCTA	AGGCCCCTCA
4251	TCCGTAGGCG	CGGCGTCCGG	GGCGTCTGCC	AGAGCGTAAG	
4301	CACTCGAGAC	CGGCAAGCCC	GTCAAAAACC CAGTTTTTGG	TCCAAAGGGG	GTACGAAAAA
4351	CTACGCAAAG	AATGGAGACC	TTTCCATGAG AAAGGTACTC	GGCCACAGGT	GCGAGCCACT
		CAGGCACAGG	GGCATATGTC	TGAACTCTCC	GGACAGGAGC
		GCGCCAGGAG	GAGCATATCT	TTGAGCCTGG	TGAGACTCTG
		CAGGTCCGGT	CGTGCTTCCT	CCGATTCACC	CTCCCCATCG
		GTGATCCCCC	AGGTGAGCGA	GGTCCCACAC	TTCTGTGTAC
4601	TCGCCCTCTT AGCGGGAGAA	CGGCATCAAG GCCGTAGTTC	GAAGGTGATŢ CTTCCACTAA	GGTTTGTAGG CCAAACATCC	TGTAGGCCAC ACATCCGGTG

Figure 27E

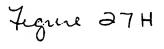
4701				CTGTTGGGGT GACAACCCCA
4751				GATTGTCAGT CTAACAGTCA
4801				GTGATGCCTT CACTACGGAA
4851				TTTGTTGTCA AAACAACAGT
4901				ACTTGGCGAT TGAACCGCTA
4951				TTGGCCGCGA AACCGGCGCT
5001			CGCGCAACGC GCGCGTTGCG	 
5051			CAGGTGCACG GTCCACGTGC	 
5101			TGGCTACCTC ACCGATGGAG	 
5151			TTGCGCGAGC AACGCGCTCG	 
5201			GTCTGCGTCC CAGACGCAGG	
5251			CTATCTTGCA GATAGAACGT	 
5301			AGCGCGCGCT TCGCGCGCGA	
5351			GAGCGCGGAG CTCGCGCCTC	 
5401	GTAAACGTAG CATTTGCATC		TGAGTATTCC ACTCATAAGG	 
5451	TTCCACCGCG AAGGTGGCGC	- · · · · - · · · · · · · · · · · · · ·		 
5501	GCGAGGAGGT CGCTCCTCCA		GTTGCTACGG CAACGATGCC	 
5551	GACTATCTGC CTGATAGACG		CATGTGAGTT GTACACTCAA	 



5651	GAGGCGTAGG CTCCGCATCC	AGTCGCGCAG TCAGCGCGTC	CTTGTTGACC GAACAACTGG	AGCTCGGCGG TCGAGCCGCC	TGACCTGCAC ACTGGACGTG
5701	GTCTAGGGCG	CAGTAGTCCA	GGGTTTCCTT CCCAAAGGAA	GATGATGTCA	TACTTATCCT
5751	GTCCCTTTTT	TTTCCACAGC	TCGCGGTTGA	GGACAAACTC	TTCGCGGTCT
3,31	CAGGGAAAAA	AAAGGTGTCG	AGCGCCAACT	CCTGTTTGAG	AAGCGCCAGA
5801	TTCCAGTACT AAGGTCATGA	CTTGGATCGG GAACCTAGCC	AAACCCGTCG TTTGGGCAGC	GCCTCCGAAC CGGAGGCTTG	CCATTCTCGG
5851	TAGCATGTAG ATCGTACATC	AACTGGTTGA TTGACCAACT	CGGCCTGGTA GCCGGACCAT	GGCGCAGCAT CCGCGTCGTA	CCCTTTTCTA GGGAAAAGAT
5901	CGGGTAGCGC	GTATGCCTGC CATACGGACG	GCGGCCTTCC CGCCGGAAGG	GGAGCGAGGT CCTCGCTCCA	GTGGGTGAGC CACCCACTCG
5951	GCAAAGGTGT	CCCTGACCAT	GACTTTGAGG	TACTGGTATT	TGAAGTCAGT
			CTGAAACTCC CCCAGAGCAA		
6001	CAGCAGCGTA	GGCGGGACGA	GGGTCTCGTT	TTTCAGGCAC	GCGAAAAACC
6051	AACGCGGATT TTGCGCCTAA	TGGCAGGGCG ACCGTCCCGC	AAGGTGACAT TTCCACTGTA	CGTTGAAGAG GCAACTTCTC	TATCTTTCCC ATAGAAAGGG
6101	GCGCGAGGCA CGCGCTCCGT	TAAAGTTGCG ATTTCAACGC	TGTGATGCGG ACACTACGCC	AAGGGTCCCG TTCCCAGGGC	GCACCTCGGA CGTGGAGCCT
6151	ACGGTTGTTA TGCCAACAAT	ATTACCTGGG TAATGGACCC	CGGCGAGCAC GCCGCTCGTG	GATCTCGTCA CTAGAGCAGT	AAGCCGTTGA TTCGGCAACT
6201	TGTTGTGGCC ACAACACCGG	CACAATGTAA GTGTTACATT	AGTTCCAAGA TCAAGGTTCT	AGCGCGGGAT TCGCGCCCTA	GCCCTTGATG CGGGAACTAC
6251	GAAGGCAATT	TTTTAAGTTC AAAATTCAAG	CTCGTAGGTG GAGCATCCAC	AGCTCTTCAG TCGAGAAGTC	GGGAGCTGAG CCCTCGACTC
6301	CCCGTGCTCT GGGCACGAGA	GAAAGGGCCC CTTTCCCGGG	AGTCTGCAAG TCAGACGTTC	ATGAGGGTTG TACTCCCAAC	GAAGCGACGA CTTCGCTGCT
6351	እጥር እርርጥር CA	CAGGTCACGG		TTTGCAGGTG	GTCGCGAAAG
6401	GTCCTAAACT CAGGATTTGA	GGCGACCTAT CCGCTGGATA	GGCCATTTTT CCGGTAAAAA	TCTGGGGTGA AGACCCCACT	TGCAGTAGAA ACGTCATCTT
6451	GGTAAGCGGG CCATTCGCCC	TCTTGTTCCC AGAACAAGGG	AGCGGTCCCA TCGCCAGGGT	TCCAAGGTTC AGGTTCCAAG	GCGGCTAGGT CGCCGATCCA
6501	CTCGCGCGGC GAGCGCGCCG	AGTCACTAGA TCAGTGATCT	GGCTCATCTC CCGAGTAGAG	CGCCGAACTT GCGGCTTGAA	CATGACCAGC GTACTGGTCG

Figure 276

6601	TACATCGTAG	GTGACAAAGA	GACGCTCGGT	GCGAGGATGC	GAGCCGATCG
	ATGTAGCATC	CACTGTTTCT	CTGCGAGCCA	CGCTCCTACG	CTCGGCTAGC
6651					ATTGATGTGG
	CCTTCTTGAC	CTAGAGGGCG	GTGGTTAACC	TCCTCACCGA	TAACTACACC
6701					GGCTTTTGTA
	ACTTTCATCT	TCAGGGACGC	TGCCCGGCTT	GTGAGCACGA	CCGAAAACAT
6751					TCCTGCACGA
					AGGACGTGCT
6801	GGTTGACCTG				
					AAACTCGGGG
6851					CTTGTCCTTG
					GAACAGGAAC
6901					ACCACGCCGC
					TGGTGCGGCG
6951					CTTGATGACA
					GAACTACTGT
7001					GCGGCGTCAG
			CAGGTACCAG		
7051			GGTTTACCTC		
			CCAAATGGAG		
7101			CTAATTTCCA		
			GATTAAAGGT		
7151			GCATCCCCGC CGTAGGGGCG		
	•				
7201	GCCGCCCGCC		GGGTGTCCTT		
	GCCGCCGCC	ACCCGGCGCC	CCCACAGGAA	CCIACIACGI	AGATTTICGC
7251	GTGACGCGGG	CGAGCCCCCG	GAGGTAGGGG	GGGCTCCGGA	CCCGCCGGGA
	CACTGCGCCC	GCTCGGGGGC	CTCCATCCCC	CCCGAGGCCT	GGGCGCCCT
7301	GAGGGGGCAG				
	CTCCCCGTC	CCCGTGCAGC	CGCGGCGCGC	GCCCGTCCTC	GACCACGACG
7351	GCGCGTAGGT				
	CGCGCATCCA	ACGACCGCTT	GCGCTGCTGC	GCCGCCAACT	AGAGGACTTA
7401	CTGGCGCCTC				
	GACCGCGGAG	ACGCACTTCT	GCTGCCCGGG	CCACTCGAAC	TTGGACTTTC
7451	AGAGTTCGAC				
	TCTCAAGCTG	TCTTAGTTAA	AGCCACAGCA	ACTGCCGCCG	GACCGCGTTT



2001	CMCCMCC NMC	TCTTCCTCCT	CCACATOTO	CCGTCCGGCT	CGCTCCACGG
7551	CACCACCTAG	AGAAGGAGGA	CCTCTAGAGG	CGCAGGCCGA	GCGAGGTGCC
	GACGAGC 1AG				
7601	TGGCGGCGAG	GTCGTTGGAA	ATGCGGGCCA	TGAGCTGCGA	GAAGGCGTTG
	ACCGCCGCTC	CAGCAACCTT	TACGCCCGGT	ACTCGACGCT	CTTCCGCAAC
					emmoocco\mc
7651	AGGCCTCCCT	CGTTCCAGAC	GCGGCTGTAG	ACCACGCCCC	CTTCGGCATC
	TCCGGAGGGA	GCAAGGTCTG	CGCCGACATC	TGGTGCGGG	GAAGCCGIAG
2201	000000000	ATGACCACCT	CCCCCACATT	GAGCTCCACG	TGCCGGGCGA
7701	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	TACTGGTGGA	CGCGCTCTAA	CTCGAGGTGC	ACGGCCCGCT
7751	AGACGGCGTA	GTTTCGCAGG	CGCTGAAAGA	GGTAGTTGAG	GGTGGTGGCG
	TCTGCCGCAT	CAAAGCGTCC	GCGACTTTCT	CCATCAACTC	CCACCACCGC
	_			0>000m0003	አርርጥርርኔጥጥር
7801	GTGTGTTCTG	CCACGAAGAA GGTGCTTCTT	GTACATAACC	CTCCCACCCT	TGCACCTAAG
	CACACAAGAC	GGTGCTTCTT	CATGIAITIGG	GICGCAGCGI	10chec 112.0
7851	CTTCATATCC	CCCAAGGCCT	CAAGGCGCTC	CATGGCCTCG	TAGAAGTCCA
7031	CAACTATAGG	GGGTTCCGGA	GTTCCGCGAG	GTACCGGAGC	ATCTTCAGGT
7901	CGGCGAAGTT	GAAAAACTGG	GAGTTGCGCG	CCGACACGGT	TAACTCCTCC
	GCCGCTTCAA	CTTTTTGACC	CTCAACGCGC	GGCTGTGCCA	ATTGAGGAGG
		GGATGAGCTC	CCCCACACTC	TOCOCOLACOT	CCCCTCAAA
7951	TCCAGAAGAC	CCTACTCGAG	CCCCACACIG	AGCGCGTGGA	GCGCGAGTTT
	AGGICITCIG	CCIACICGAG	2001010		
8001	GGCTACAGGG	GCCTCTTCTT	CTTCTTCAAT	CTCCTCTTCC	ATAAGGGCCT
5001	CCGATGTCCC	CGGAGAAGAA	GAAGAAGTTA	GAGGAGAAGG	TATTCCCGGA
8051	CCCCTTCTTC	TTCTTCTGGC	GGCGGTGGGG	GAGGGGGGAC	ACGGCGGCGA
	GGGGAAGAAG	AAGAAGACCG	CCGCCACCCC	Crecectic	1900000001
0101	CCACCCCCA	CCGGGAGGCG	GTCGACAAAG	CGCTCGATCA	TCTCCCCGCG
8101	CCACGCGCA	GCCCTCCGC	CAGCTGTTTC	GCGAGCTAGT	AGAGGGGCGC
8151	GCGACGGCGC	ATGGTCTCGG	TGACGGCGCG	GCCGTTCTCG	CGGGGGCGCA
	CGCTGCCGCG	TACCAGAGCC	ACTGCCGCGC	CGGCAAGAGC	GCCCCCGCGT
	_		1 = C = C C C C C C	ma mocoomyco	CCCCCCCCTC
8201	GTTGGAAGAC	GCCGCCCGTC CGGCGGGCAG	TACACCCCCA	ATACCCAACC	GCCCCCGAC
	CAACCTTCTG	CGGCGGGCMG	IACAGGGCCA	AIACCCITICE	••••
8251	CCATGCGGCA	GGGATACGGC	GCTAACGATG	CATCTCAACA	ATTGTTGTGT
0231	GGTACGCCGT	CCCTATGCCG	CGATTGCTAC	GTAGAGTTGT	TAACAACACA
8301	AGGTACTCCG	CCGCCGAGGG	ACCTGAGCGA	GTCCGCATCG	ACCGGATCGG
	TCCATGAGGC	GGCGGCTCCC	TGGACTCGCT	CAGGCGTAGC	TGGCCTAGCC
		C>C>>>>	ጥርጥን አርርንርጥ	ראראפתרפרא	AGGTAGGCTG
B351	AAAACCTCTC	クリンクヤイヤイシャン フリンクヤイヤイシャン	AGATTGGTCA	GTGTCAGCGT	TCCATCCGAC
	1-1-1-1-GOVGWG				
8401	AGCACCGTGG	CGGGCGGCAG	CGGCGGCGG	TCGGGGTTGT	TTCTGGCGGA
	TCGTGGCACC	GCCCGCCGTC	GCCCGCCGCC	AGCCCCAACA	AAGACCGCCT

Figure 27I

8501					GCGCAGGCGG CGCGTCCGCC
8551					CTTTGTAGTA GAAACATCAT
8601	• • • • • • • • • • • • • • • • • • • •				TCCTCTTGTC AGGAGAACAG
8651		TGCATCTATC ACGTAGATAG			TGGCCGTAGG ACCGGCATCC
8701		TTCCTCCCAT AAGGAGGGTA			TCATCGGCTG AGTAGCCGAC
8751		AGGTCGGCGA TCCAGCCGCT			GCCTGCTGCA CGGACGACGT
8801		GGTAGACTGG CCATCTGACC			
8851		TGATGGTGTA ACTACCACAT			
8901	••••	CCCGGCTGCG GGGCCGACGC			
8951		AAATACGTAG TTTATGCATC			
9001		AGTGCGGCGG TCÁCGCCGCC			
9051		CCGGGGGCGA GGCCCCCGCT			
9101		GGACATCCAG CCTGTAGGTC			
9151	CCTTTCAGCG	GGACGCGGTT CCTGCGCCAA	GGTCTACAAC	CCGTCGCCGT	TTTTCACGAG
9201		ACGCTCTGGC TGCGAGACCG			TTGACGCTCT. AACTGCGAGA
9251	AGACCGTGCA TCTGGCACGT	AAAGGAGAGC TTTCCTCTCG			
9301	GGATAAATTC CCTATTTAAG	GCAAGGGTAT CGTTCCCATA			
9351	ATCCGGCCGT TAGGCCGGCA	CCGCCGTGAT GGCGGCACTA			

Figure 27J

9451	CCGCGCCGCC	CTGCTGCGCT GACGACGCGA	AGCTTTTTTG TCGAAAAAAC	GCCACTGGCC CGGTGACCGG	GCGCGCAGCG CGCGCGTCGC
9501	TAAGCGGTTA	GGCTGGAAAG	CGAAAGCATT	AAGTGGCTCG	CTCCCTGTAG
	ATTCGCCAAT	CCGACCTTTC	GCTTTCGTAA	TTCACCGAGC	GAGGGACATC
9551	CCGGAGGGTT	ATTTTCCAAG	GGTTGAGTCG	CGGGACCCCC	GGTTCGAGTC
	GGCCTCCCAA	TAAAAGGTTC	CCAACTCAGC	GCCCTGGGGG	CCAAGCTCAG
9601	TCGGACCGGC	CGGACTGCGG	CGAACGGGGG	TTTGCCTCCC	CGTCATGCAA
	AGCCTGGCCG	GCCTGACGCC	GCTTGCCCCC	AAACGGAGGG	GCAGTACGTT
9651	GACCCCGCTT	GCAAATTCCT	CCGGAAACAG	GGACGAGCCC	CTTTTTTGCT
	CTGGGGCGAA	CGTTTAAGGA	GGCCTTTGTC	CCTGCTCGGG	GAAAAAACGA
9701	TTTCCCAGAT	GCATCCGGTG	CTGCGGCAGA	TGCGCCCCCC	TCCTCAGCAG
	AAAGGGTCTA	CGTAGGCCAC	GACGCCGTCT	ACGCGGGGGG	AGGAGTCGTC
9751	CGGCAAGAGC	AAGAGCAGCG	GCAGACATGC	AGGGCACCCT	CCCCTCCTCC
	GCCGTTCTCG	TTCTCGTCGC	CGTCTGTACG	TCCCGTGGGA	GGGGAGGAGG
9801	TACCGCGTCA	GGAGGGGCGA	CATCCGCGGT	TGACGCGGCA	GCAGATGGTG
	ATGGCGCAGT	CCTCCCCGCT	GTAGGCGCCA	ACTGCGCCGT	CGTCTACCAC
9851	ATTACGAACC TAATGCTTGG	CCCGCGCGCG	CCCCCCCCC	ACTACCTGGA TGATGGACCT	CTTGGAGGAG GAACCTCCTC
9901	GGCGAGGGCC	TGGCGCGGCT	AGGAGCGCCC	TCTCCTGAGC	GGCACCCAAG
	CCGCTCCCGG	ACCGCGCCGA	TCCTCGCGGG	AGAGGACTCG	CCGTGGGTTC
9951	GGTGCAGCTG	AAGCGTGATA	CGCGTGAGGC	GTACGTGCCG	CGGCAGAACC
	CCACGTCGAC	TTCGCACTAT	GCGCACTCCG	CATGCACGGC	GCCGTCTTGG
10001	TGTTTCGCGA	CCGCGAGGGA	GAGGAGCCCG	AGGAGATGCG	GGATCGAAAG
	ACAAAGCGCT	GGCGCTCCCT	CTCCTCGGGC	TCCTCTACGC	CCTAGCTTTC
10051	TTCCACGCAG	GGCGCGAGCT	GCGGCATGGC	CTGAATCGCG	AGCGGTTGCT
	AAGGTGCGTC	CCGCGCTCGA	CGCCGTACCG	GACTTAGCGC	TCGCCAACGA
10101	GCGCGAGGAG	GACTTTGAGC	CCGACGCGCG	AACCGGGATT	AGTCCCGCGC
	CGCGCTCCTC	CTGAAACTCG	GGCTGCGCGC	TTGGCCCTAA	TCAGGGCGCG
10151	GCGCACACGT CGCGTGTGCA	CCCCCCCCC	GACCTGGTAA CTGGACCATT	CCGCATACGA GGCGTATGCT	GCAGACGGTG CGTCTGCCAC
10201	AACCAGGAGA	TTAACTTTCA	AAAAAGCTTT	AACAACCACG	TGCGTACGCT
	TTGGTCCTCT	AATTGAAAGT	TTTTTCGAAA	TTGTTGGTGC	ACGCATGCGA
10251	TGTGGCGCGC	GAGGAGGTGG	CTATAGGACT	GATGCATCTG	TGGGACTTTG
	ACACCGCGCG	CTCCTCCACC	GATATCCTGA	CTACGTAGAC	ACCCTGAAAC
10301	TAAGCGCGCT	GGAGCAAAAC	CCAAATAGCA	AGCCGCTCAT	GGCGCAGCTG
	ATTCGCGCGA	CCTCGTTTTG	GGTTTATCGT	TCGGCGAGTA	CCGCGTCGAC

Figure 27K

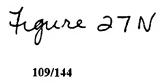
10401					TTGATAAACA AACTATTTGT
10451				GCTTGAGCCT CGAACTCGGA	GGCTGACAAG CCGACTGTTC
10501				CTGGGCAAGT GACCCGTTCA	TTTACGCCCG AAATGCGGGC
10551			<del>-</del>	AGACAAGGAG TCTGTTCCTC	
10601				TGCTTACCTT ACGAATGGAA	
10651				AAGGCCGTGA TTCCGGCACT	
10701				GCACAGCCTG CGTGTCGGAC	
10751				CCGAGTCCTA GGCTCAGGAT	
10801				CGCGCCCTGG GCGCGGGACC	
10851	CCGGCCTGGA	CCCGACCGCC	ACCGTGGGCG	GCGCGCTGGC CGCGCGACCG	TTGCAGCCGC
10901				ACGAGCCAGA TGCTCGGTCT	
10951	ATGATTCGCC	ACTACAAAGA	CTAGTCTACT	TGCAAGACGC ACGTTCTGCG	TTGCCTGGGC
11001	CGCCACGCCC	GCCGCGACGT	CTCGGTCGGC	TCCGGCCTTA AGGCCGGAAT	TGAGGTGCCT
11051				GTCGCTGACT CAGCGACTGA	
11101	CTGACGCGTT GACTGCGCAA				
11151	GAAGCGGTGG CTTCGCCACC				
11201	GATCGTAAAC CTAGCATTTG				
11251	GCCTGGTCTA CGGACCAGAT				

Figure 27L

11351	GGCGCAGCGT CCGCGTCGCA	GAGCGCGCGC CTCGCGCGCG	AGCAGCAGGG TCGTCGTCCC	CAACCTGGGC GTTGGACCCG	TCCATGGTTG AGGTACCAAC
11401	CACTAAACGC GTGATTTGCG	CTTCCTGAGT GAAGGACTCA	ACACAGCCCG TGTGTCGGGC	CCAACGTGCC GGTTGCACGG	GCGGGGACAG CGCCCCTGTC
11451	CTCCTGATGT	GGTTGAAACA	CTCGCGTGAC	CGGCTAATGG GCCGATTACC	ACTGACTCTG
11501	TGGCGTTTCA	CTCCACATGG	TCAĢACCCGG	AGACTATTTT TCTGATAAAA	AAGGTCTGGT
11551	CATCTGTTCC	GGACGTCTGG	CATTTGGACT	GCCAGGCTTT CGGTCCGAAA	GTTTTTGAAC
11601	GTCCCCGACA	CCCCCCACGC	CCGAGGGTGT	GGCGACCGCG CCGCTGGCGC	GCTGGCACAG
11651	ATCGAACGAC	TGCGGGTTGA	GCGCGGACAA	GCTGCTGCTA CGACGACGAT	TATCGCGGGA
11701	AGTGCCTGTC	ACCGTCGCAC	AGGGCCCTGT	CATACCTAGG GTATGGATCC	AGTGAACGAC
11751	TGTGACATGG	CGCTCCGGTA	TCCAGTCCGC	CATGTGGACG GTACACCTGC	TCGTATGAAA
11801	GGTCCTCTAA	TGTTCACAGT	CGGCGCGCGA	GGGGCAGGAG CCCCGTCCTC	CTGTGCCCGT
11851	CGGACCTCCG	TTGGGATTTG	ATGGACGACT	CCAACCGGCG GGTTGGCCGC	CGTCTTCTAG
11901	GGGAGCAACG	TGTCAAATTT	GTCGCTCCTC	GAGCGCATTT CTCGCGTAAA	ACGCGATGCA
11951	CGTCGTCTCG	CACTCGGAAT	TGGACTACGC	CGACGGGGTA GCTGCCCCAT	TGCGGGTCGC
12001	ACCGCGACCT	GTACTGGCGC	GCGTTGTACC	AACCGGGCAT TTGGCCCGTA	CATACGGAGT
	TTGGCCGGCA	AATAGTTGGC	GGATTACCTG	ATGAACGTAG	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
	GCACTTGGGG	CTCATAAAGT	GGTTACGGTA	GAACTTGGGC	CACTGGCTAC GTGACCGATG
	GCGGGGGACC	AAAGATGTGG	CCCCCTAAGC	TCCACGGGCT	GGGTAACGAT CCCATTGCTA
12201	GGATTCCTCT CCTAAGGAGA	GGGACGACAT CCCTGCTGTA	AGACGACAGC TCTGCTGTCG	GTGTTTTCCC CACAAAAGGG	CGCAACCGCA GCGTTGGCGT

Figure 27 M

12301					CGCTGCGGCC
	TCCTTTCGAA	GGCGTCCGG1	TCGTCGAACA	GGCTAGATCC	GCGACGCCGG
12351					GGTCTCTTAC
	GGCGCCAGTC	TACGATCATC	GGGTAAAGGT	TCGAACTATC	CCAGAGAATG
12401					GAGTACCTAA
	GTCGTGAGCG	TGGTGGGCGG	GCGCGGACGA	CCCGCTCCTC	CTCATGGATT
12451					TCCGGCATTT
	TGTTGAGCGA	CGACGTCGGC	GTCGCGCTTI	TTTTGGACGG	AGGCCGTAAA
12501					GATGGAAGAC
	GGGTTGTTGC	CCTATCTCTC	GGATCACCTG	TTCTACTCAT	CTACCTTCTG
12551					CCCACCCGTC
	CATGCGCGTC	CTCGTGTCCC	TGCACGGTCC	GGGCGCGGC	GGGTGGGCAG
12601					CGATGACTCG
	CAGTTTCCGT	GCTGGCAGTC	GCCCCAGACC	ACACCCTCCT	GCTACTGAGC
12651				GGGAGTGGCA	
	CGTCTGCTGT	CGTCGCAGGA	CCTAAACCCT	CCCTCACCGT	TGGGCAAACG
12701	GCACCTTCGC	CCCAGGCTGG	GGAGAATGTT	TTAAAAAAAA	AAAAAGCATG
	CGTGGAAGCG	GGGTCCGACC	CCTCTTACAA	AATTTTTTTT	TTTTTCGTAC
12751				GCACCGAGCG	
	TACGTTTTAT	TTTTTGAGTG	GTTCCGGTAC	CGTGGCTCGC	AACCAAAAGA
12801				ATGTATGAGG	
	ACATAAGGGG	AATCATACGC	CGCGCGCCGC	TACATACTCC	TTCCAGGAGG
12851				GCCAGTGGCG	
	AGGGAGGATG	CTCTCACACC	ACTCGCGCCG	CGGTCACCGC	CGCCGCGACC
12901	GTTCTCCCTT	CGATGCTCCC	CTGGACCCGC	CGTTTGTGCC	TCCGCGGTAC
	CAAGAGGGAA	GCTACGAGGG	GACCTGGGCG	GCAAACACGG	AGGCGCCATG
12951				CGTTACTCTG	
	GACGCCGGAT	GGCCCCCCTC	TTTGTCGTAG	GCAATGAGAC	TCAACCGTGG
13001	CCTATTCGAC	ACCACCCGTG	TGTACCTGGT	GGACAACAAG	TCAACGGATG
	GGATAAGCTG	TGGTGGGCAC	ACATGGACCA	CCTGTTGTTC	AGTTGCCTAC
13051	TGGCATCCCT	GAACTACCAG	AACGACCACA	GCAACTTTCT	GACCACGGTC
	ACCGTAGGGA	CTTGATGGTC	TTGCTGGTGT	CGTTGAAAGA	CTGGTGCCAG
13101	ATTCAAAACA				
	TAAGTTTTGT	TACTGATGTC	GGGCCCCCTC	CGTTCGTGTG	TCTGGTAGTT
13151	TCTTGACGAC	CGGTCGCACT	GGGGCGGCGA	CCTGAAAACC	ATCCTGCATA
	AGAACTGCTG	GCCAGCGTGA	CCCCGCCGCT	GGACTTTTGG	TAGGACGTAT



13251	CGGGTGATGG	TGTCGCGCTT	GCCTACTAAG	GACAATCAGG	TGGAGCTGAA
	GCCCACTACC	ACAGCGCGAA	CGGATGATTC	CTGTTAGTCC	ACCTCGACTT
13301	ATACGAGTGG	GTGGAGTTCA	CGCTGCCCGA	GGGCAACTAC	TCCGAGACCA
	TATGCTCACC	CACCTCAAGT	GCGACGGGCT	CCCGTTGATG	AGGCTCTGGT
13351	TGACCATAGA	CCTTATGAAC	AACGCGATCG	TGGAGCACTA	CTTGAAAGTG
	ACTGGTATCT	GGAATACTTG	TTGCGCTAGC	ACCTCGTGAT	GAACTTTCAC
13401	GGCAGACAGA	ACGGGGTTCT	GGAAAGCGAC	ATCGGGGTAA	AGTTTGACAC
	CCGTCTGTCT	TGCCCCAAGA	CCTTTCGCTG	TAGCCCCATT	TCAAACTGTG
13451	CCGCAACTTC	AGACTGGGGT	TTGACCCCGT	CACTGGTCTT	GTCATGCCTG
	GGCGTTGAAG	TCTGACCCCA	AACTGGGGCA	GTGACCAGAA	CAGTACGGAC
13501	GGGTATATAC	AAACGAAGCC	TTCCATCCAG	ACATCATTTT	GCTGCCAGGA
	CCCATATATG	TTTGCTTCGG	AAGGTAGGTC	TGTAGTAAAA	CGACGGTCCT
13551	TGCGGGGTGG	ACTTCACCCA	CAGCCGCCTG	AGCAACTTGT	TGGGCATCCG
	ACGCCCCACC	TGAAGTGGGT	GTCGGCGGAC	TCGTTGAACA	ACCCGTAGGC
13601	CAAGCGGCAA	CCCTTCCAGG	AGGGCTTTAG	GATCACCTAC	GATGATCTGG
	GTTCGCCGTT	GGGAAGGTCC	TCCCGAAATC	CTAGTGGATG	CTACTAGACC
13651	AGGGTGGTAA	CATTCCCGCA	CTGTTGGATG	TGGACGCCTA	CCAGGCGAGC
	TCCCACCATT	GTAAGGGCGT	GACAACCTAC	ACCTGCGGAT	GGTCCGCTCG
13701	TTGAAAGATG	ACACCGAACA	GGGCGGGGGT	GGCGCAGGCG	GCAGCAACAG
	AACTTTCTAC	TGTGGCTTGT	CCCGCCCCCA	CCGCGTCCGC	CGTCGTTGTC
13751	CAGTGGCAGC GTCACCGTCG	GGCGCGGAAG CCGCGCCTTC	AGAACTCCAA TCTTGAGGTT	CGCGGCAGCC	GCGGCAATGC CGCCGTTACG
13801	AGCCGGTGGA	GGACATGAAC	GATCATGCCA	TTCGCGGCGA	CACCTTTGCC
	TCGGCCACCT	CCTGTACTTG	CTAGTACGGT	AAGCGCCGCT	GTGGAAACGG
13851	ACACGGGCTG	AGGAGAAGCG	CGCTGAGGCC	GAAGCAGCGG	CCGAAGCTGC
	TGTGCCCGAC	TCCTCTTCGC	GCGACTCCGG	CTTCGTCGCC	GGCTTCGACG
13901	CGCCCCGCT	GCGCAACCCG	AGGTCGAGAA	GCCTCAGAAG	AAACCGGTGA
	GCGGGGGCGA	CGCGTTGGGC	TCCAGCTCTT	CGGAGTCTTC	TTTGGCCACT
13951	TCAAACCCCT	GACAGAGGAC	AGCAAGAAAC	GCAGTTACAA	CCTAATAAGC
	AGTTTGGGGA	CTGTCTCCTG	TCGTTCTTTG	CGTCAATGTT	GGATTATTCG
14001	AATGACAGCA	CCTTCACCCA	GTACCGCAGC	TGGTACCTTG	CATACAACTA
	TTACTGTCGT	GGAAGTGGGT	CATGGCGTCG	ACCATGGAAC	GTATGTTGAT
14051	CGGCGACCCT	CAGACCGGAA	TCCGCTCATG	GACCCTGCTT	TGCACTCCTG
	GCCGCTGGGA	GTCTGGCCTT	AGGCGAGTAC	CTGGGACGAA	ACGTGAGGAC
14101	ACGTAACCTG	CGGCTCGGAG	CAGGTCTACT	GGTCGTTGCC	AGACATGATG
	TGCATTGGAC	GCCGAGCCTC	GTCCAGATGA	CCAGCAACGG	TCTGTACTAC

Tigure 270

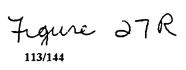
14201					TACAACGACC
	CCACCCGCGC	G CTCGACAACG	GGCACGTGA	G GTTCTCGAA	ATGTTGCTGG
14251	AGGCCGTCTA	CTCCCAACTC	ATCCGCCAG	TTACCTCTCT	GACCCACGTG
	TCCGGCAGAT	GAGGGTTGAG	TAGGCGGTC	A AATGGAGAGA	CTGGGTGCAC
14301	TTCAATCGCT	TTCCCGAGAA	CCAGATTTT	G GCGCGCCGG	CAGCCCCCAC
	AAGTTAGCGA	AAGGGCTCTT	GGTCTAAAA	CGCGCGGGC	GTCGGGGGTG
14351	CATCACCACC	GTCAGTGAAA	ACGTTCCTG	TCTCACAGAT	CACGGGACGC
	GTAGTGGTGG	CAGTCACTTT	TGCAAGGACO	AGAGTGTCTA	GTGCCCTGCG
14401	TACCGCTGCG	CAACAGCATC	GGAGGAGTCC	AGCGAGTGAC	CATTACTGAC
	ATGGCGACGC	GTTGTCGTAG	CCTCCTCAGG	TCGCTCACTG	GTAATGACTG
14451	GCCAGACGCC	GCACCTGCCC	CTACGTTTAC	AAGGCCCTGG	GCATAGTCTC
	CGGTCTGCGG	CGTGGACGGG	GATGCAAATG	TTCCGGGACC	CGTATCAGAG
14501					TCCATCCTTA
		GATAGCTCGG			
14551	TATCGCCCAG	CAATAACACA	GGCTGGGGCC	TGCGCTTCCC	AAGCAAGATG
		GTTATTGTGT			
14601	TTTGGCGGGG	CCAAGAAGCG	CTCCGACCAA	CACCCAGTGC	GCGTGCGCGG
		GGTTCTTCGC			
14651		GCGCCCTGGG			
		CGCGGGACCC			
14701	CCACCGTCGA	TGACGCCATC	GACGCGGTGG	TGGAGGAGGC	GCGCAACTAC
		ACTGCGGTAG			
14751	ACGCCCACGC	CGCCACCAGT	GTCCACAGTG	GACGCGGCCA	TTCAGACCGT
	•	GCGGTGGTCA			
14801	GGTGCGCGGA	GCCCGGCGCT	ATGCTAAAAT	GAAGAGACGG	CGGAGGCGCG
14051		CGGGCCGCGA			
14851	ARCACGICG	CCACCGCCGC	CGACCCGGCA	CTGCCGCCCA	ACGCGCGGCG
		GGTGGCGGCG			
14901	GCGGCCCTGC	TTAACCGCGC	ACGTCGCACC	GGCCGACGGG	CGGCCATGCG
		AATTGGCGCG	•		
14951	GGCCGCTCGA				
	CCGGCGAGCT				
15001	GGCGACGAGC	GGCCGCCGCA	GCAGCCGCGG	CCATTAGTGC	TATGACTCAG
	CCGCTGCTCG				
15051	GGTCGCAGGG	GCAACGTGTA '	TTGGGTGCGC	GACTCGGTTA	GCGGCCTGCG
	CCAGCGTCCC	CGTTGCACAT I	AACCCACGCG	CTGAGCCAAT	CGCCGGACGC



15151	ACTTAGACTC TGAATCTGAG	GTACTGTTGT CATGACAACA	ATGTATCCAG TACATAGGTC	GCCGCCGCCGC	GCGCAACGAA CGCGTTGCTT
15201	GCTATGTCCA CGATACAGGT	AGCGCAAAAT TCGCGTTTTA	CAAAGAAGAG GTTTCTTCTC	ATGCTCCAGG TACGAGGTCC	TCATCGCGCC AGTAGCGCGG
15251	GGAGATCTAT CCTCTAGATA	GGCCCCCGA CCGGGGGGCT	AGAAGGAAGA TCTTCCTTCT	GCAGGATTAC CGTCCTAATG	AAGCCCCGAA TTCGGGGCTT
15301	AGCTAAAGCG TCGATTTCGC	GGTCAAAAAG CCAGTTTTTC	AAAAAGAAAG TTTTTCTTTC	ATGATGATGA TACTACTACT	TGAACTTGAC ACTTGAACTG
15351	GACGAGGTGG CTGCTCCACC	AACTGCTGCA TTGACGACGT	CGCTACCGCG GCGATGGCGC	CCCAGGCGAC GGGTCCGCTG	GGGTACAGTG CCCATGTCAC
15401	CTTTCCAGCT	GCGCATTTTG	GTGTTTTGCG CACAAAACGC	TGGGCCGTGG	TGGCATCAGA
15451	AATGCGGGCC	ACTCGCGAGG	ACCCGCACCT TGGGCGTGGA	TGTTCGCGCA	CATACTACTC
15501	CACATGCCGC	TGCTCCTGGA	GCTTGAGCAG CGAACTCGTC	CGGTTGCTCG	CGGAGCCCCT
15551	CAAACGGATG	CCTTTCGCCG	ATAAGGACAT TATTCCTGTA	CGACCGCAAC	GGCGACCTGC
15601	TCCCGTTGGG	TTGTGGATCG	CTAAAGCCCG GATTTCGGGC	ATTGTGACGT	CGTCCACGAC
15651	GGGCGCGAAC	GTGGCAGGCT	AGAAAAGCGC TCTTTTCGCG	CCGGATTTCG	CGCTCAGACC
15701	ACTGAACCGT	GGGTGGCACG	AGCTGATGGT TCGACTACCA	TGGGTTCGCG	GTCGCTGACC
15751	TTCTACAGAA	CCTTTTTTAC	ACCGTGGAAC TGGCACCTTG	GACCCGACCT	CGGGCTCCAG
15801	GCGCACGCCG	GTTAGTTCGT	CCACCGCGGC	CCTGACCCGC	
	CCTGCAAGTC	TATGGGTGAT	GGTCATCGTG	GTCATAACGG	ACCGCCACAG TGGCGGTGTC
	TCCCGTACCT	CTGTGTTTGC	AGGGGCCAAC	GGAGTCGCCA	GGCGGATGCC CCGCCTACGG
	CGCCACGTCC	GCCAGCGACG	CCGGCGCAGG	TTCTGGAGAT	CGGAGGTGCA GCCTCCACGT
16001	AACGGACCCG TTGCCTGGGC	TGGATGTTTC	GCGTTTCAGC GCGCAAAGTCG	CCCCCGGCGC	CCGCGCCGTT

Figure 270

16051				TGCCCGAATA ACGGGCTTAT	
16101				GGCTACACCT CCGATGTGGA	
16151				CACTGGAACC GTGACCTTGG	
16201				TTTCCGTGCG AAAGGCACGC	
16251				ACAGCGCGCT TGTCGCGCGA	
16301			<del>-</del>	TGCAGATATG ACGTCTATAC	
16351				GAGGAAGAAT CTCCTTCTTA	
16401				GGCATGCGTC CCGTACGCAG	
16451		•		GCGCGGCGGT CGCGCCGCCA	
16501			•	GCGCCGTGCC CGCGGCACGG	
16551				TTAAAAACAA AATTTTTGTT	
16601				ACGCTCGCTT TGCGAGCGAA	
16651			•	GCGTCTCTGG CGCAGAGACC	
16701	CGGCTCGCGC GCCGAGCGCG			AGATATCGGC TCTATAGCCG	
16751	TGAGCGGTGG ACTCGCCACC			TGTGGAGCGG ACACCTCGCC	
16801	TTCGGTTCCA AAGCCAAGGT			AAGGCCTGGA TTCCGGACCT	
16851	AGGCCAGATG TCCGGTCTAC			GCAAAATTTC CGTTTTAAAG	
16901	TGGTAGATGG ACCATCTACC			GGGTGGTGGA CCCACCACCT	
16951	CAGGCAGTGC GTCCGTCACG			CTTGATCCCC GAACTAGGGG	



17051	AAAAGCGTCC	GCGCCCCGAC	AGGGAAGAAA	CTCTGGTGAC	GCAAATAGAC
	TTTTCGCAGG	CGCGGGGCTG	TCCCTTCTTT	GAGACCACTG	CGTTTATCTG
17161	GAGCCTCCCT	CGTACGAGGA	GGCACTAAAG	CAAGGCCTGC	CCACCACCCG
	CTCGGAGGGA	GCATGCTCCT	CCGTGATTTC	GTTCCGGACG	GGTGGTGGGC
17151	TCCCATCGCG	CCCATGGCTA	CCGGAGTGCT	GGGCCAGCAC	ACACCCGTAA
	AGGGTAGCGC	GGGTACCGAT	GGCCTCACGA	CCCGGTCGTG	TGTGGGCATT
17201	CGCTGGACCT	GCCTCCCCC	GCCGACACCC	AGCAGAAACC	TGTGCTGCCA
	GCGACCTGGA	CGGAGGGGGG	CGGCTGTGGG	TCGTCTTTGG	ACACGACGGT
17251				AGCCGCGCGT TCGGCGCGCA	
17301	CGCCGCCAGC	GGTCCGCGAT	CGTTGCGGCC	CGTAGCCAGT	GGCAACTGGC
	GCGGCGGTCG	CCAGGCGCTA	GCAACGCCGG	GCATCGGTCA	CCGTTGACCG
17351				GGGTGCAATC CCCACGTTAG	
17401	CGACGATGCT	TCTGATAGCT	AACGTGTCGT	ATGTGTGTCA	TGTATGCGTC
	GCTGCTACGA	AGACTATCGA	TTGCACAGCA	TACACACAGT	ACATACGCAG
17451	CATGTCGCCG GTACAGCGGC	CCAGAGGAGC GGTCTCCTCG	TGCTGAGCCG ACGACTCGGC	GCGCGCGCCC	GCTTTCCAAG CGAAAGGTTC
17501	ATGGCTACCC	CTTCGATGAT	GCCGCAGTGG	TCTTACATGC	ACATCTCGGG
	TACCGATGGG	GAAGCTACTA	CGGCGTCACC	AGAATGTACG	TGTAGAGCCC
17551	CCAGGACGCC	TCGGAGTACC	TGAGCCCCGG	GCTGGTGCAG	TTTGCCCGCG
	GGTCCTGCGG	AGCCTCATGG	ACTCGGGGCC	CGACCACGTC	AAACGGGCGC
17601	CCACCGAGAC	GTACTTCAGC	CTGAATAACA	AGTTTAGAAA	CCCCACGGTG
	GGTGGCTCTG	CATGAAGTCG	GACTTATTGT	TCAAATCTTT	GGGGTGCCAC
17651	GCGCCTACGC	ACGACGTGAC	CACAGACCGG	TCCCAGCGTT	TGACGCTGCG
	CGCGGATGCG	TGCTGCACTG	GTGTCTGGCC	AGGGTCGCAA	ACTGCGACGC
17701	GTTCATCCCT	GTGGACCGTG	AGGATACTGC	GTACTCGTAC	AAGGCGCGGT
	CAAGTAGGGA	CACCTGGCAC	TCCTATGACG	CATGAGCATG	TTCCGCGCCA
17751	TCACCCTAGC	TGTGGGTGAT	AACCGTGTGC	TGGACATGGC	TTCCACGTAC
	AGTGGGATCG	ACACCCACTA	TTGGCACACG	ACCTGTACCG	AAGGTGCATG
17801	TTTGACATCC	GCGGCGTGCT	GGACAGGGGC	CCTACTTTTA	AGCCCTACTC
	AAACTGTAGG	CGCCGCACGA	CCTGTCCCCG	GGATGAAAAT	TCGGGATGAG
17851	TGGCACTGCC ACCGTGACGG	TACAACGCCC ATGTTGCGGG	TGGCTCCCAA ACCGAGGGTT	GGGTGCCCCA	AATCCTTGCG TTAGGAACGC
17901	AATGGGATGA	AGCTGCTACT	GCTCTTGAAA	TAAACCTAGA	AGAAGAGGAC
	TTACCCTACT	TCGACGATGA	CGAGAACTTT	ATTTGGATCT	TCTTCTCCTG

Figure 275

17951					AAAAAACTCA TTTTTTGAGT
18001				AAATATTACA TTTATAATGT	AAGGAGGGTA TTCCTCCCAT
18051				AATATGCCGA TTATACGGCT	TAAAACATTT ATTTTGTAAA
18101				TGGTACGAAA ACCATGCTTT	
18151		•		TACCCCAATG ATGGGGTTAC	
18201				ATGGAGGGCA TACCTCCCGT	
18251				CAAGTGGAAA GTTCACCTTT	
18301			•	TGATAACTTG ACTATTGAAC	
18351				AAACCCCAGA TTTGGGGTCT	
18401	TCTTACATGC AGAATGTACG			TCACGAGAAC AGTGCTCTTG	
18451		• •		TGCTTTTAGG ACGAAAATCC	
18501			-	ATATGGGTGT TATACCCACA	
18551		-		TTGCAAGACA AACGTTCTGT	
18601				TGGTGATAGA ACCACTATCT	
18651	TTTCTATGTG AAAGATACAC				· ·
18701	ATTGAAAATC TAACTTTTAG				
18751	GGGAGGTGTG CCCTCCACAC				
18801	GTCAGGAAAA CAGTCCTTTT				
18851	GAAATAAGAG CTTTATTCTC				

Figure 27T

18951	AGCTAAAGTA TCGATTTCAT	CAGTCCTTCC GTCAGGAAGG	AACGTAAAAA TTGCATTTTT	TTTCTGATAA AAAGACTATT	CCCAAACACC GGGTTTGTGG
19001		TGAACAAGCG ACTTGTTCGC			
19051	CATTAACCTT GTAATTGGAA	GGAGCACGCT CCTCGTGCGA	GGTCCCTTGA CCAGGGAACT	CTATATGGAC GATATACCTG	AACGTCAACC TTGCAGTTGG
19101	GTAAATTGGT	CCACCGCAAT GGTGGCGTTA	CGACCGGACG	CGATGGCGAG	TTACAACGAC
19151	CCGTTACCAG	GCTATGTGCC CGATACACGG	GAAGGTGTAG	GTCCACGGAG	TCTTCAAGAA
19201	ACGGTAATTT	AACCTCCTTC TTGGAGGAAG	AGGACGGCCC	GAGTATGTGG	ATGCTCACCT
19251	TGAAGTCCTT	GGATGTTAAC CCTACAATTG	TACCAAGACG	TCTCGAGGGA	TCCTTTACTG
19301	GATTCCCAAC	ACGGAGCCAG TGCCTCGGTC	GTAATTCAAA	CTATCGTAAA	CGGAAATGCG
19351	GTGGAAGAAG	CCCATGGCCC GGGTACCGGG	TGTTGTGGCG	GAGGTGCGAA	CTCCGGTACG
19401	AATCTTTGCT	CACCAAÇGAC GTGGTTGCTG	GTCAGGAAAT	TGCTGATAGA	GAGGCGGCGG
19451	TTGTACGAGA	ACCCTATACC TGGGATATGG	GCGGTTGCGA	TGGTTGCACG	GGTATAGGTA
19501	GGGGAGGCG	AACTGGGCGG TTGACCCGCC	GAAAGGCGCC	GACCCGGAAG	TGCGCGGAAT
19551	TCTGATTCCT	AACCCCATCA TTGGGGTAGT	GACCCGAGCC	CGATGCTGGG	AATAATGTGG
19601	ATGAGACCGA	CTATACCCTA GATATGGGAT	GGATCTACCT	TGGAAAATGG	AGTTGGTGTG
		CACCGGTAAT	GGAAACTGAG	AAGACAGTCG	ACCGGACCGT
		CGAATGGGGG	TTGCTCAAAC	TTTAATTCGC	GAGTCAACTG
		TGTTGCAACG	GGTCACATTG	TACTGGTTTC	TGACCAAGGA
19801	GGTACAAATG CCATGTTTAC	CTAGCTAACT GATCGATTGA	ATAACATTGG TATTGTAACC	CTACCAGGGC GATGGTCCCG	AAGATATAGG

Figure 274

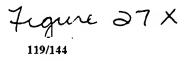
19851				CTTCCAGCCC GAAGGTCGGG
19901				ACCAACAGGT TGGTTGTCCA
19951				TACCTTGCCC ATGGAACGGG
20001				CTATCCGCTT GATAGGCGAA
20051				TTCTTTGCGA AAGAAACGCT
20101				 TCCATGGGCG AGGTACCCGC
20151				CGCCCACGCG GCGGGTGCGC
20201				 CCCTTCTTTA GGGAAGAAAT
20251		GAAGTCTTTG CTTCAGAAAC		 CCGCACCGCGGGGGCGC
20301		AACCGTGTAC TTGGCACATG		 
20351		GAAGCAAGCA CTTCGTTCGT		
20401		AACTGAAAGC TTGACTTTCG		 
20451		ACCTATGACA TGGATACTGT		
20501		CGCCATAGTC GCGGTATCAG		 
20551	CACTGGATGG GTGACCTACC			 
20601	TGAGCCCTTT ACTCGGGAAA			 
20651	AGTACGAGTC TCATGCTCAG			
20701	TGTATAACGC ACATATTGCG	=	-	 
20751	CGCCTGTGGA GCGGACACCT			 

Figure 27 V.

20851	CCCAACTCCA GGGTTGAGGT	TGCTCAACAG ACGAGTTGTC	TCCCCAGGTA AGGGGTCCAT	CAGCCCACCC GTCGGGTGGG	TGCGTCGCAA ACGCAGCGTT
20901	CCAGGAACAG GGTCCTTGTC	CTCTACAGCT GAGATGTCGA	TCCTGGAGCG AGGACCTCGC	CCACTCGCCC GGTGAGCGGG	TACTTCCGCA ATGAAGGCGT
20951	CGGTGTCACG	GCAGATTAGG CGTCTAATCC	TCGCGGTGAA	GAAAAACAGT	GAACTTTTTG
21001	TACATTTTTA	AATGTACTAG TTACATGATC	TCTGTGAAAG	TTATTTCCGT	TTACGAAAAT
21051	AAACATGTGA	CTCGGGTGAT GAGCCCACTA	ATAAATGGGG	GTGGGAACGG	CAGACGCGGC
21101		TTTCCCCAAG	ACGGCGCGTA	GCGATACGCG	GTGACCGTCC
21151	CTGTGCAACG	GATACTGGTG CTATGACCAC	AAATCACGAG	GTGAATTTGA	GTCCGTGTTG
21201	GTAGGCGCCG	AGCTCGGTGA TCGAGCCACT	TCAAAAGTGA	GGTGTCCGAC	GCGTGGTAGT
21251	GGTTGCGCAA	TAGCAGGTCG ATCGTCCAGC	CCGCGGCTAT	AGAACTTCAG	CGTCAACCCC
21301	GGAGGCGGGA	CGCGCGCGCT	CAACGCTATG	TGTCCCAACG	
21351	GTGATAGTCG	CGGCCCACCA	CGTGCGACCG	GTCGTGCGAG	
21401	AGTCTAGGCG	CAGGTCCAGG	AGGCGCAACG	AGTCCCGCTT	CGGAGTCAAC GCCTCAGTTG
21451	AAACCATCGA	CGGAAGGGTT	TTTCCCGCGC	ACGGGTCCGA	TTGAGTTGCA AACTCAACGT
21501	GAGCGTGGCA	TCACCGTAGT	TTTCCACTGG	CACGGGCCAG	TGGGCGTTAG ACCCGCAATC
	CTATGTCGCG	GACGTATTTT	CGGAACTAGA	CGAATTTTCG	CACCTGAGCC GTGGACTCGG
	AAACGCGGAA	GTCTCTTCTT	GTACGGCGTT	CTGAACGGCC	AAAACTGATT TTTTGACTAA
	CCGGCCTGTC	CGGCGCAGCA	CGTGCGTCGI	GGAACGCAGC	GTGTTGGAGA CACAACCTCT
21701	TCTGCACCAC AGACGTGGTG	TAAAGCCGGG	CACCGGTTCT GTGGCCAAGA	TCACGATCTI AGTGCTAGAA	GGCCTTGCTA CCGGAACGAT

7. gure 27 W

21801	AATCACGTG	C TCCTTATTT	A TCATAATGC	T TCCGTGTAG	A CACTTAAGCT
	TTAGTGCAC	G AGGAATAAA1	r agtattacg	A AGGCACATC	r gtgaattcga
21051	000000000				
21851					A GCCCGTGGGC CGGGCACCCG
	GCGGAAGC 17	A GAGTEGEGTE	. GCCACGTCG	G TGTTGCGCGT	r CGGGCACCCG
21901	TCGTGATGCT	TGTAGGTCAC	CTCTGCAAA	C GACTGCAGGI	ACGCCTGCAG
	AGCACTACGA	ACATCCAGTG	GAGACGTTT	G CTGACGTCCA	TGCGGACGTC
21951					AAGGTCAGCT
	CTTAGCGGGG	TAGTAGCAGT	GTTTCCAGA	A CAACGACCAC	TTCCAGTCGA
22001	GCAACCCGCG	: הייהריירריירה	የተመር አርርር አርር	~ <del>~~~~~</del> ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	GGCCGCCAGA
22001	CGTTGGGCGC	CACGAGGAGC	AAGTCGGTC	AGAACGTATG	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
22051	GCTTCCACTT	GGTCAGGCAG	TAGTTTGAAG	TTCGCCTTTA	GATCGTTATC
	CGAAGGTGAA	CCAGTCCGTC	ATCAAACTTC	: AAGCGGAAAT	CTAGCAATAG
	0.0000000				
22101				AGCCTCCATG	
	GIGCACCAIG	AACAGGTAGT	CGCGCGCGC	TCGGAGGTAC	GGGAAGAGGG
22151	ACGCAGACAC	GATCGGCACA	CTCAGCGGGT	TCATCACCGT	ልል <b>ጥጥጥ</b> ለ ርጭጥ
				AGTAGTGGCA	
22201				TGCGTCCGCA	
	AGGCGAAGCG	ACCCGAGAAG	GAGAAGGAGA	ACGCAGGCGT	ATGGTGCGCG
22251	C	memme a mme a	66666666	TGTGCGCTTA	
22231				ACACGCGAAT	
	010.000.00	1107010172101	000000000	ACACGCGAAI	GGAGGAAACG
22301	CATGCTTGAT	TAGCACCGGT	GGGTTGCTGA	AACCCACCAT	TTGTAGCGCC
	GTACGAACTA	ATCGTGGCCA	CCCAACGACT	TTGGGTGGTA	AACATCGCGG
22351	ACATCTTCTC	TTTCTTCCTC	GCTGTCCACG	ATTACCTCTG	GTGATGGCGG
	TGTAGAAGAG	AAAGAAGGAG	CGACAGGTGC	TAATGGAGAC	CACTACCGCC
22401	GCGCTCGGGC	TTGGGAGAAG	GGCGCTTCTT	TTTCTTCTTG	GGCGC A ATCC
				AAAGAAGAAC	
22451	CCAAATCCGC	CGCCGAGGTC	GATGGCCGCG	GGCTGGGTGT	GCGCGGCACC
	GGTTTAGGCG	GCGGCTCCAG	CTACCGGCGC	CCGACCCACA	CGCGCCGTGG
22501	>00000mama	GMC1 MC1 GMC	######################################		
22301	TOCOCCOACAA	CACTACTCAC	AACCACCACC	TCGGACTCGA AGCCTGAGCT	TACGCCGCCT
	rededendana	CACIACICAG	ANGGAGÇAGG	AGCCTGAGCT	ATGUGGUGGA
22551	CATCCGCTTT	TTTGGGGGCG	CCCGGGGAGG	CGGCGGCGAC	GGGGACGGGG
•				GCCGCCGCTG	
22601				GCGCCGCACC	
	TGCTGTGCAG	GAGGTACCAA	CCCCCTGCAG	CGCGGCGTGG	CGCAGGCGCG
22551	שרבכרבבשבב -	<b>ずずずんししししゅし</b>		001000000	MMMA
22001				CGACTGGCCA GCTGACCGGT	
		. www. coconc	CHOCHOCH	GCIGACCGGT	AAAUUAAGAG

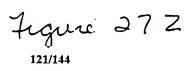


PCT/US01/28861

22751	CCGCCCCCTC	TGAGTTCGCC	ACCACCGCCT	CCACCGATGC	CGCCAACGCG
	GGCGGGGGAG	ACTCAAGCGG	TGGTGGCGGA	GGTGGCTACG	GCGGTTGCGC
	00000000				
22801	ССТАССАССТ	TCCCCGTCGA	GGCACCCCCG	CTTGAGGAGG	AGGAAGTGAT
22001	CCIACCACCI	ACCCCCACCA	CCGTGGGGGC	GAACTCCTCC	TCCTTCACTA
	GGATGGTGGA	AGGGGCAGC I	CCG1GGGGGC	0,2,0100100	
00053	m> moo> co>c	これのことれてこので	<b>ጥጥርጥል አርርርል</b>	AGACGACGAG	GACCGCTCAG
22851				TCTGCTGCTC	
	ATAGCTCGTC	CIGGICCAA	AACATICGCI	1010010010	¢1000d1.0.0
			CAACACCACC	ACAACGCAGA	CCCYAACGAG
22901	TACCAACAGA	GGATAAAAAG	CAAGACCAGG	MCMMCCCMCM	CCGTTTCCTC
	ATGGTTGTCT	CCTATTTTC	Griciegicc	TGTTGCGTCT	CCG111GC1C
			0011100017	GGCGACTACC	тасатетесе
22951	GAACAAGTCG	GGCGGGGGA	CGAAAGGCAT	GGCGACTACC	17C72C2CC
	CTTGTTCAGC	CCGCCCCCT	GCTTTCCGTA	CCGCTGATGG	KICIACACCC
				50×0000000	» mm» mdmccc
23001	AGACGACGTG	CTGTTGAAGC	ATCTGCAGCG	CCAGTGCGCC	MINICIGEG
	TCTGCTGCAC	GACAACTTCG	TAGACGTCGC	GGTCACGCGG	TAATAGACGC
					001 00001 00
23051	ACGCGTTGCA	AGAGCGCAGC	GATGTGCCCC	TCGCCATAGC	GGATGTCAGC
	TGCGCAACGT	TCTCGCGTCG	CTACACGGGG	AGCGGTATCG	CCTACAGTCG
					~~~~~
23101	CTTGCCTACG	AACGCCACCT	ATTCTCACCG	CGCGTACCCC	CCAAACGCCA
	GAACGGATGC	TTGCGGTGGA	TAAGAGTGGC	GCGCATGGGG	GGTTTGCGGT
23151	AGAAAACGGC	ACATGCGAGC	CCAACCCGCG	CCTCAACTTC	TACCCCGTAT
	TCTTTTGCCG	TGTACGCTCG	GGTTGGGCGC	GGAGTTGAAG	ATGGGGCATA
23201	TTGCCGTGCC	AGAGGTGCTT	GCCACCTATC	ACATCTTTTT	CCAAAACTGC
	AACGGCACGG	TCTCCACGAA	CGGTGGATAG	TGTAGAAAAA	GGTTTTGACG
23251	AAGATACCCC	TATCCTGCCG	TGCCAACCGC	AGCCGAGCGG	ACAAGCAGCT
	TTCTATGGGG	ATAGGACGGC	ACGGTTGGCG	TCGGCTCGCC	TGTTCGTCGA
23301	GGCCTTGCGG	CAGGGCGCTG	TCATACCTGA	TATCGCCTCG	CTCAACGAAG
	CCGGAACGCC	GTCCCGCGAC	AGTATGGACT	ATAGCGGAGC	GAGTTGCTTC
23351				ACGAGAAGCG	
	ACGGTTTTTA	GAAACTCCCA	GAACCTGCGC	TGCTCTTCGC	GCGCCGTTTG
23401	GCTCTGCAAC	AGGAAAACAG	CGAAAATGAA	AGTCACTCTG	GAGTGTTGGT
	CGAGACGTTG	TCCTTTTGTC	GCTTTTACTT	TCAGTGAGAC	CTCACAACCA
23451	GGAACTCGAG	GGTGACAACG	CGCGCCTAGC	CGTACTAAAA	CGCAGCATCG
	CCTTGAGCTC	CCACTGTTGC	GCGCGGATCG	GCATGATTTT	GCGTCGTAGC
23501	AGGTCACCCA	CTTTGCCTAC	CCGGCACTTA	ACCTACCCCC	CAAGGTCATG
	TCCAGTGGGT	GAAACGGATG	GGCCGTGAAT	TGGATGGGG	GTTCCAGTAC
23551	AGCACAGTCA	TGAGTGAGCT	GATCGTGCGC	CGTGCGCAGC	CCCTGGAGAG
2,,,,	TCGTGTCAGT	ACTCACTCGA	CTAGCACGCG	GCACGCGTCG	GGGACCTCTC
	1001010H01				
23601	GGATGCAAAT	TTGCAAGAAC	AAACAGAGGA	GGGCCTACCC	GCAGTTGGCG
25001	CCTACCTOTA	AACGTTCTTG	TTTGTCTCCT	CCCGGATGGG	CGTCAACCGC
	CCINCUITIN	,			

Figure 27 Y

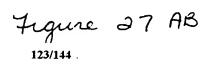
23701				CTCGTTACCG GAGCAATGGC	
23751				GATGCAGCGC CTACGTCGCG	
23801				ACGTACGCCA TGCATGCGGT	
23851			•••	TCCTACCTTG AGGATGGAAC	•
23901	•			TTCCACGCTC AAGGTGCGAG	
23951				ACTTATTTCT TGAATAAAGA	
24001				TGCTTGGAGG ACGAACCTCC	
24051				CTTGAAGGAC GAACTTCCTG	
24101		•		TGGCGGACAT ACCGCCTGTA	
24151			• •	CTGCCAGACT GACGGTCTGA	
24201				CCTAGAGCGC GGATCTCGCG	-
24251				ACTTTGTGCC TGAAACACGG	
24301	CGCGAATGCC GCGCTTACGG			TGCTACCTTC ACGATGGAAG	
24351			•	GGAAGACGTG CCTTCTGCAC	
24401	GTCTACTGGA CAGATGACCT			TATGCACCCC ATACGTGGGG	
24451	CTGGTTTGCA GACCAAACGT			AGTCAAATTA TCAGTTTAAT	
24501	TGAGCTGCAG ACTCGACGTC			GTCCGCGGCT CAGGCGCCGA	
24551	AACTCACTCC TTGAGTGAGG			ACCTTCGCAA TGGAAGCGTT	



24601	GAGGACTACC	ACCCCCACGA	GATTAGGTTC	TACGAAGACC	AATCCCGCCC
24001	ondone ince	TGCGGGTGCT	CDAADCCAAC	* INCOMPONO	TTACCCCCC
	CTCCTGATGG	10CGG1GC1	CIAAICCAAG	AIGCIICIGG	114000000
24651	GCCTAATGCG	GAGCTTACCG	CCTGCGTCAT	TACCCAGGGC	CACATTCTTG
		CTCGAATGGC			
	COOMITACOC	C1C0381.00C	ouncocnor		
24701	GCCAATTGCA				
	CGGTTAACGT	TCGGTAGTTG	TTTCGGGCGG	TTCTCAAAGA	CGATGCTTTC
		TTTACTTGGA	000000000000	CCCCACCACC	TO A A COCA AT
24751					
	CCTGCCCCCC	AAATGAACCT	GGGGGTCAGG	CCGCTCCTCG	AGTIGGGTTA
24801	concoggeg	CCGCAGCCCT	ATCAGCAGCA	GCCGCGGGCC	CTTGCTTCCC
• • • • • • • • • • • • • • • • • • • •		GGCGTCGGGA			
	666666666	GOCGICGGGA	INGICUICGI	COGCOCCCOO	G12.CG12.GGG
24851	AGGATGGCAC	CCAAAAAGAA	GCTGCAGCTG	CCGCCGCCAC	CCACGGACGA
	TCCTACCGTG	GGTTTTTCTT	CGACGTCGAC	GCCGCCGGTG	GGTGCCTGCT
		TGGGACAGTC	100010100	CCDDDDCCAC	CACCACCACC
24901					
	CCTCCTTATG	ACCCTGTCAG	TCCGTCTCCT	CCAAAACCTG	CTCCTCCTCC
24951	ACCACATGAT	GGAAGACTGG	GAGAGCCTAG	ACGAGGAAGC	TTCCGAGGTC
24331	MOGACATOAT	CCTTCTGACC	COCOCCATC	TOCTOCTTCC	AAGGCTCCAG
	TCCTGTACTA	CCTTCTGACC	CICICGGAIC	1001001100	ANGGETEENG
25001		CAGACGAAAC			
	CTTCTCCACA	GTCTGCTTTG	TGGCAGTGGG	AGCCAGCGTA	AGGGGAGCGG
				•	
				C> #CCC#\C\	N COMPCCCOMC
25051		AAATCGGCAA			
	CCGCGGGGTC	TTTAGCCGTT	GGCCAAGGTC	GTACCGATGT	TGGAGGCGAG
			•		
25101	CECACCCCC	GCCGGCACTG	CCCGTTCGCC	GACCCAACCG	TAGATGGGAC
23101		CGGCCGTGAC			
	GAGTCCGCGG	CGGCCGTGAC	GGGCAAGCGG	C16661166C	AICIACCCIG
25151	ACCACTGGAA	CCAGGGCCGG	TAAGTCCAAG	CAGCCGCCGC	CGTTAGCCCA
	TCCTCACCTT	GGTCCCGGCC	ATTCAGGTTC	GTCGGCGGCG	GCAATCGGGT
	10010110011				
				*********	CNCNNCNNCC
25201		CAGCGCCAAG			
	TCTCGTTGTT	GTCGCGGTTC	CGATGGCGAG	TACCGCGCCC	GTGTTCTTGC
25251	CCATAGTTGC	TTGCTTGCAA	GACTGTGGGG	GCAACATCTC	CTTCGCCCGC
23231	CCAIAGIIGC	AACGAACGTT	CECACACCCC	CCTTCTACAC	CANCCCCCCC
	GGTATCAACG	AACGAACGTT	CIGACACCCC	CGIIGIAGAG	GAAGCGGGCG
25301	CGCTTTCTTC	TCTACCATCA	CGGCGTGGCC	TTCCCCCGTA	ACATCCTGCA
	GCGAAAGAAG	AGATGGTAGT	GCCGCACCGG	AAGGGGGCAT	TGTAGGACGT
	000.22.0.2.0				
			0000181080	a.aaaaaaaa	*CCCCC*CC*
25351	TTACTACCGT	CATCTCTACA	GCCCATACTG	CALLGGLGGL	AGCGGCAGCA
	AATGATGGCA	GTAGAGATGT	CGGGTATGAC	GTGGCCGCCG	TCGCCGTCGT
25/01	ACAGCAGCGG	CCACACAGAA	CCAAACCCCA	CCGGATAGCA	AGACTCTGAC
<b>7340T</b>	ACAGCAGCGG		COMMISSION	CCCCTATCCT	ጥርጥርልርአርጥር
	TGTCGTCGCC	GGTGTGTCTT	CGTTTCCGCT	GOCCIMICGI.	TCIGNGACIG
25451	AAAGCCCAAG	AAATCCACAG	CGGCGGCAGC	AGCAGGAGGA	GGAGCGCTGC
	MANUCCCUMUC	TTTAGGTGTC	CCCCCCCCTCC	TOGTOCTOCT	CCTCGCGACG
	111000110	nogiGiC	2223663166		
					****
25501	GTCTGGCGCC	CAACGAACCC	GTATCGACCC	GCGAGCTTAG	AAACAGGATT
	CAGACCGCGG	GTTGCTTGGG	CATAGCTGGG	CGCTCGAATC	TTTGTCCTAA

Figure 27 AA

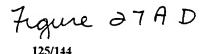
25551				AGCAGGGGCC	
	AAAGGGTGAG	ACATACGATA	TAAAGTTGTC	TCGTCCCCGG	TTCTTGTTC
25601	GCTGAAAATA	AAAAACAGGT	CTCTGCGATC	CCTCACCCGC	AGCTGCCTG
	CGACTTTTAT	TTTTTGTCCA	GAGACGCTAG	GGAGTGGGCG	TCGACGGAC
25651				CGCTGGAAGA	
	TAGTGTTTTC	GCTTCTAGTC	GAAGCCGCGT	GCGACCTTCT	GCGCCTCCG
25701	-			AAGGACTAGT	
	GAGAAGTCAT	TTATGACGCG	CGACTGAGAA	TTCCTGATCA	AAGCGCGGG
25751	TTCTCAAATT	TAAGCGCGAA	AACTACGTCA	TCTCCAGCGG	CCACACCCG
				AGAGGTCGCC	
25801	CGCCAGCACC	TGTTGTCAGC	GCCATTATGA	GCAAGGAAAT	TCCCACGCCC
				CGTTCCTTTA	
25851	TACATGTGGA	GTTACCAGCC	ACAAATGGGA	CTTGCGGCTG	GAGCTGCCCA
				GAACGCCGAC	
25901	AGACTACTCA	ACCCGAATAA	ACTACATGAG	CGCGGGACCC	CACATGATAT
	TCTGATGAGT	TGGGCTTATT	TGATGTACTC	GCGCCCTGGG	GTGTACTATA
25951	CCCGGGTCAA	CGGAATACGC	GCCCACCGAA	ACCGAATTCT	CCTGGAACAG
				TGGCTTAAGA	
26001	GCGGCTATTA	CCACCACACC	TCGTAATAAC	CTTAATCCCC	GTAGTTGGCC
				GAATTAGGGG	
26051	CGCTGCCCTG	GTGTACCAGG	AAAGTCCCGC	TCCCACCACT	GTGGTACTTC
	GCGACGGGAC	CACATGGTCC	TTTCAGGGCG	ÄGGGTGGTGA	CACCATGAAG
26101	CCAGAGACGC	CCAGGCCGAA	GTTCAGATGA	CTAACTCAGG	GGCGCAGCTT
	GGTCTCTGCG	GGTCCGGCTT	CAAGTCTACT	GATTGAGTCC	CCGCGTCGAA
26151	GCGGGCGGCT	TTCGTCACAG	GGTGCGGTCG	CCCGGGCAGG	GTATAACTCA
		-		GGGCCCGTCC	
26201	CCTGACAATC	AGAGGGCGAG	GTATTCAGCT	CAACGACGAG	TCGGTGAGCT
				GTTGCTGCTC	
26251	CCTCGCTTGG	TCTCCGTCCG	GACGGGACAT	TTCAGATCGG	CGCCCCGGC
				AAGTCTAGCC	
26301	CGCTCTTCAT	TCACGCCTCG	TCAGGCAATC	CTAACTCTGC	AGACCTCGTC
20301	GCGAGAAGTA				
26351	CTCTGAGCCG	CGCTCTGGAG	GCATTGGAAC	TCTGCAATTT	ATTGAGGAGT
•	GAGACTCGGC				
26401	TTGTGCCATC	GGTCTACTTT	AACCCCTTCT	CGGGACCTCC	CGGCCACTAT
	AACACGGTAG				
26451	CCGGATCAAT	TTATTCCTAA	CTTTGACGCG	GTAAAGGACT	CGGCGGACGG
	CCCCTACTTA				



26501	CTACGACTGA	ATGTTAAGTG	GAGAGGCAGA	GCAACTGCGC	CTGAAACACC
	GATGCTGACT	TACAATTCAC	CTCTCCGTCT	CGTTGACGCG	GACTTTGTGG
26551	TGGTCCACTG	TCGCCGCCAC	AAGTGCTTTG	CCCGCGACTC	CGGTGAGTTT
	ACCAGGTGAC	AGCGGCGGTG	TTCACGAAAC	GGGCGCTGAG	GCCACTCAAA
26601	TGCTACTTTG	AATTGCCCGA	GGATCATATC	GAGGGCCCGG	CGCACGGCGT
			CCTAGTATAG		
26651	CCGGCTTACC	GCCCAGGGAG	AGCTTGCCCG	TAGCCTGATT	CGGGAGTTTA
			TCGAACGGGC		
26701	CCCAGCGCCC	CCTGCTAGTT	GAGCGGGACA	GGGGACCCTG	TGTTCTCACT
_			CTCGCCCTGT		
26751	GTGATTTGCA	ACTGTCCTAA	CCCTGGATTA	CATCAAGATC	TTTGTTGCCA
		•	GGGACCTAAT		
26801	TCTCTGTGCT	GAGTATAATA	AATACAGAAA	TTAAAATATA	CTGGGGCTCC
	-		TTATGTCTTT		
26851	TATCGCCATC	CTGTAAACGC	CACCGTCTTC	ACCCGCCCAA	GCAAACCAAG
			GTGGCAGAAG		
26901	GCGAACCTTA	CCTGGTACTT	TTAACATCTC	TCCCTCTGTG	ATTTACAACA
			AATTGTAGAG		
26951	GTTTCAACCC	AGACGGAGTG	AGTCTACGAG	AGAACCTCTC	CGAGCTCAGC
			TCAGATGCTC		
27001	TACTCCATCA	GAAAAAACAC	CACCCTCCTT	ACCTGCCGGG	AACGTACGAG
			GTGGGAGGAA		
27051	TGCGTCACCG	GCCGCTGCAC	CACACCTACC	GCCTGACCGT	AAACCAGAC1
			GTGTGGATGG		
27101	TTTTCCGGAC	AGACCTCAAT	AACTCTGTTT	ACCAGAACAG	GAGGTGAGCT
			TTGAGACAAA		
27151	TAGAAAACCC	TTAGGGTATT	AGGCCAAAGG	CGCAGCIACI	GTGGGGTTTA
			TCCGGTTTCC		
27201	TGAACAATTC	AAGCAACTCT	ACGGGCTATT	CAMTAACTC	TTTCTCTAGA
•					AAAGAGATCT
27251	ATCGGGGTTG	GGGTTATTCT	CTGTCTTGTG	ATTOTOTA	TTCTTATACT
					AAGAATATGA
27301	AACGCTTCTC	TGCCTAAGGC	TCGCCGCCTG	CIGIGIGIAC	ATTTGCATTT
					TAAACGTAAA
27351	ATTGTCAGCT	TTTTAAACGC	TGGGGTCGCC	ACCCAAGATG	ATTAGGTACA
			•		TAATCCATGT
27401	TAATCCTAGG	TTTACTCACC	CTTGCGTCAG	CCCACGGTAC	CACCCAAAAG
	ATTAGGATCO	AAATGAGTGG	GAACGCAGTC	GGGTGCCATG	GTGGGTTTTC

Figure 27AC

WO 02/022080 PCT/US01/28861 27451 GTGGATTTTA AGGAGCCAGC CTGTAATGTT ACATTCGCAG CTGAAGCTAA CACCTAAAAT TCCTCGGTCG GACATTACAA TGTAAGCGTC GACTTCGATT 27501 TGAGTGCACC ACTCTTATAA AATGCACCAC AGAACATGAA AAGCTGCTTA · ACTCACGTGG TGAGAATATT TTACGTGGTG TCTTGTACTT TTCGACGAAT 27551 TTCGCCACAA AAACAAAATT GGCAAGTATG CTGTTTATGC TATTTGGCAG AAGCGGTGTT TTTGTTTTAA CCGTTCATAC GACAAATACG ATAAACCGTC 27601 CCAGGTGACA CTACAGAGTA TAATGTTACA GTTTTCCAGG GTAAAAGTCA GGTCCACTGT GATGTCTCAT ATTACAATGT CAAAAGGTCC CATTTTCAGT 27651 TAAAACTTTT ATGTATACTT TTCCATTTTA TGAAATGTGC GACATTACCA ATTTTGAAAA TACATATGAA AAGGTAAAAT ACTTTACACG CTGTAATGGT 27701 TGTACATGAG CAAACAGTAT AAGTTGTGGC CCCCACAAAA TTGTGTGGAA ACATGTACTC GTTTGTCATA TTCAACACCG GGGGTGTTTT AACACACCTT 27751 AACACTGGCA CTTTCTGCTG CACTGCTATG CTAATTACAG TGCTCGCTTT TTGTGACCGT GAAAGACGAC GTGACGATAC GATTAATGTC ACGAGCGAAA 27801 GGTCTGTACC CTACTCTATA TTAAATACAA AAGCAGACGC AGCTTTATTG CCAGACATGG GATGAGATAT AATTTATGTT TTCGTCTGCG TCGAAATAAC 27851 AGGAAAAGAA AATGCCTTAA TTTACTAAGT TACAAAGCTA ATGTCACCAC TCCTTTTCTT TTACGGAATT AAATGATTCA ATGTTTCGAT TACAGTGGTG 27901 TAACTGCTTT ACTCGCTGCT TGCAAAACAA ATTCAAAAAG TTAGCATTAT ATTGACGAAA TGAGCGACGA ACGTTTTGTT TAAGTTTTTC AATCGTAATA 27951 AATTAGAATA GGATTTAAAC CCCCCGGTCA TTTCCTGCTC AATACCATTC TTAATCTTAT CCTAAATTTG GGGGGCCAGT AAAGGACGAG TTATGGTAAG 28001 CCCTGAACAA TTGACTCTAT GTGGGATATG CTCCAGCGCT ACAACCTTGA GGGACTTGTT AACTGAGATA CACCCTATAC GAGGTCGCGA TGTTGGAACT 28051 AGTCAGGCTT CCTGGATGTC AGCATCTGAC TTTGGCCAGC ACCTGTCCCG TCAGTCCGAA GGACCTACAG TCGTAGACTG AAACCGGTCG TGGACAGGGC 28101 CGGATTTGTT CCAGTCCAAC TACAGCGACC CACCCTAACA GAGATGACCA GCCTAAACAA GGTCAGGTTG ATGTCGCTGG GTGGGATTGT CTCTACTGGT 28151 ACACAACCAA CGCGGCCGCC GCTACCGGAC TTACATCTAC CACAAATACA TGTGTTGGTT GCGCCGGCGG CGATGGCCTG AATGTAGATG GTGTTTATGT 28201 CCCCAAGTTT CTGCCTTTGT CAATAACTGG GATAACTTGG GCATGTGGTG GGGGTTCAAA GACGGAAACA GTTATTGACC CTATTGAACC CGTACACCAC 28251 GTTCTCCATA GCGCTTATGT TTGTATGCCT TATTATTATG TGGCTCATCT CAAGAGGTAT CGCGAATACA AACATACGGA ATAATAATAC ACCGAGTAGA 28301 GCTGCCTAAA GCGCAAACGC GCCCGACCAC CCATCTATAG TCCCATCATT CGACGGATTT CGCGTTTGCG CGGGCTGGTG GGTAGATATC AGGGTAGTAA



28351 GTGCTACACC CAAACAATGA TGGAATCCAT AGATTGGACG GACTGAAACA

CACGATGTGG GTTTGTTACT ACCTTAGGTA TCTAACCTGC CTGACTTTGT

28451	TTTTATATTA	CTGACCCTTG	TTGCGCTTTT	TTGTGCGTGC	TCCACATTGG
	AAAATATAAT	GACTGGGAAC	AACGCGAAAA	AACACGCACG	AGGTGTAACC
28501	CTGCGGTTTC	TCACATCGAA	GTAGACTGCA	TTCCAGCCTT	CACAGTCTAT
	GACGCCAAAG	AGTGTAGCTT	CATCTGACGT	AAGGTCGGAA	GTGTCAGATA
28551	TTGCTTTACG	GATTTGTCAC	CCTCACGCTC	ATCTGCAGCC	TCATCACTGT
	AACGAAATGC	CTAAACAGTG	GGAGTGCGAG	TAGACGTCGG	AGTAGTGACA
28601	GGTCATCGCC	TTTATCCAGT	GCATTGACTG	GGTCTGTGTG	CGCTTTGCAT
	CCAGTAGCGG	AAATAGGTCA	CGTAACTGAC	CCAGACACAC	GCGAAACGTA
28651	ATCTCAGACA	CCATCCCCAG	TACAGGGACA	GGACTATAGC	TGAGCTTCTT
	TAGAGTCTGT	GGTAGGGGTC	ATGTCCCTGT	CCTGATATCG	ACTCGAAGAA
28701	AGAATTCTTT	AATTATGAAA	TTTACTGTGA	CTTTTCTGCT	GATTATTTGC
	TCTTAAGAAA	TTAATACTTT	AAATGACACT	GAAAAGACGA	CTAATAAACG
28751	ACCCTATCTG	CGTTTTGTTC	CCCGACCTCC	AAGCCTCAAA	GACATATATC
	TGGGATAGAC	GCAAAACAAG	GGGCTGGAGG	TTCGGAGTTT	CTGTATATAG
28801	ATGCAGATTC	ACTCGTATAT	GGAATATTCC	AAGTTGCTAC	AATGAAAAA
	TACGTCTAAG	TGAGCATATA	CCTTATAAGG	TTCAACGATG	TTACTTTTTT
28851	GCGATCTTTC	CGAAGCCTGG	TTATATGCAA	TCATCTCTGT	TATGGTGTTC
	CGCTAGAAAG	GCTTCGGACC	AATATACGTT	AGTAGAGACA	ATACCACAAG
28901	TGCAGTACCA	TCTTAGCCCT	AGCTATATAT	CCCTACCTTG	ACATTGGCTG
	ACGTCATGGT	AGAATCGGGA	TCGATATATA	GGGATGGAAC	TGTAACCGAC
28951	GAACGCAATA	GATGCCATGA	ACCACCCAAC	TTTCCCCGCG	CCCGCTATGC
	CTTGCGTTAT	CTACGGTACT	TGGTGGGTTG	AAAGGGGCGC	GGGCGATACG
29001	TTCCACTGCA	ACAAGTTGTT	GCCGCCGCT	TTGTCCCAGC	CAATCAGCCT
	AAGGTGACGT	TGTTCAACAA	CGGCCGCCGA	AACAGGGTCG	GTTAGTCGGA
29051	CGCCCACCTT	CTCCCACCC	CACTGAAATC	AGCTACTTTA	ATCTAACAGG
	GCGGGTGGAA	GAGGGTGGGG	GTGACTTTAG	TCGATGAAAT	TAGATTGTCC
29101	AGGAGATGAC	TGACACCCTA	GATCTAGAAA	TGGACGGAAT	TATTACAGAG
	TCCTCTACTG	ACTGTGGGAT	CTAGATCTTT	ACCTGCCTTA	ATAATGTCTC
29151	CAGCGCCTGC	TAGAAAGACG	CAGGGCAGCG	GCCGAGCAAC	AGCGCATGAA
	GTCGCGGACG	ATCTTTCTGC	GTCCCGTCGC	CGGCTCGTTG	TCGCGTACTT
29201	TCAAGAGCTC	CAAGACATGG	TTAACTTGCA	CCAGTGCAAA	AGGGGTATCT
	AGTTCTCGAG	GTTCTGTACC	AATTGAACGT	GGTCACGTTT	TCCCCATAGA
29251	TTTGTCTCGT	AAAGCAGGCC	AAAGTCACCT	ACGACAGTAA	TACCACCGGA
	AAACAGAGCA	TTTCGTCCGG	TTTCAGTGGA	TGCTGTCATT	ATGGTGGCCT
29301	CACCGCCTTA	GCTACAAGTT	GCCAACCAAG	CGTCAGAAAT	TGGTGGTCAT
	GTGGCGGAAT	CGATGTTCAA	CGGTTGGTTC	GCAGTCTTTA	ACCACCAGTA

Figure 27 A E

29401			AGGATCTCTG TCCTAGAGAC	
29451			CCCTTTAACT	
			GGGAAATTGA	
29501			TTAGCAAATT AATCGTTTAA	
29551			CAGCTCTGGT	
23331			 GTCGAGACCA	
29601			AAATGGAATG TTTACCTTAC	
29651			 TCATGTTGTT AGTACAACAA	
29701			CCCGTGTATC GGGCACATAG	
29751			 TACTCCTCCC ATGAGGAGGG	
29801			TACTCTCTTT ATGAGAGAAA	
29851			GCGCTCAAAA CGCGAGTTTT	
29901			CTCCCAAAAT GAGGGTTTTA	
29951			ACATAAACCT TGTATTTGGA	
30001	GCACCCCTCA CGTGGGGAGT		ACTGTGGCTG TGACACCGAC	
30051			GCAATCACAG CGTTAGTGTC	
30101	CCGTGCACGA GGCACGTGCT			
30151	TCAGAAGGAA AGTCTTCCTT	-	 	
	TAGCAGTACC ATCGTCATGG		 	
30251	GTAGCTTGGG CATCGAACCC			

Figure 27 AF

30351	TTTGACCGTA	GCAACTGGTC	CAGGTGTGAC	TATTAATAAT	ACTTCCTTGC
	AAACTGGCAT	CGTTGACCAG	GTCCACACTG	ATAATTATTA	TGAAGGAACG
30401	AAACTAAAGT	TACTGGAGCC	TTGGGTTTTG	ATTCACAAGG	CAATATGCAA
	TTTGATTTCA	ATGACCTCGG	AACCCAAAAC	TAAGTGTTCC	GTTATACGTT
30451	CTTAATGTAG	CAGGAGGACT	AAGGATTGAT	TCTCAAAACA	GACGCCTTAT
J0131		GTCCTCCTGA			
30501	ACTTGATGTT	AGTTATCCGT	TTGATGCTCA	AAACCAACTA	AATCTAAGAC
	TGAACTACAA	TCAATAGGCA	AACTACGAGT	TTTGGTTGAT	TTAGATTCTG
30551	TAGGACAGGG	CCCTCTTTTT	ATAAACTCAG	CCCACAACTT	GGATATTAAC
		GGGAGAAAA			
30601	TACAACAAAG	GCCTTTACTT	GTTTACAGCT	TCAAACAATT	CCAAAAAGCT
		CGGAAATGAA			•
30651	TGAGGTTAAC	CTAAGCACTG	CCAAGGGGTT	GATGTTTGAC	GCTACAGCCA
		GATTCGTGAC			
30701	TAGCCATTAA	TGCAGGAGAT	GGGCTTGAAT	TTGGTTCACC	TAATGCACCA
		ACGTCCTCTA			
30751	AACACAAATC	CCCTCAAAAC	AAAAATTGGC	CATGGCCTAG	AATTTGATTC
		GGGAGTTTTG			
30801	AAACAAGGCT	ATGGTTCCTA	AACTAGGAAC	TGGCCTTAGT	TTTGACAGCA
•		TACCAAGGAT	•		
30851	CAGGTGCCAT	TACAGTAGGA	AACAAAAATA	ATGATAAGCT	AACTTTGTGG
		ATGTCATCCT			
30901	ACCACACCAG	CTCCATCTCC	TAACTGTAGA	CTAAATGCAG	AGAAAGATGC
		GAGGTAGAGG			
30951	TAAACTCACT	TTGGTCTTAA	CAAAATGTGG	CAGTCAAATA	CTTGCTACAG
		AACCAGAATT			
31001	TTTCAGTTTT	GGCTGTTAAA	GGCAGTTTGG	CTCCAATATC	TGGAACAGTT
		CCGACAATTT			
31051	CAAAGTGCTC	ATCTTATTAT	AAGATTTGAC	GAAAATGGAG	TGCTACTAAA
	GTTTCACGAG				
31101	CAATTCCTTC	CTGGACCCAG	AATATTGGAA	CTTTAGAAAT	GGAGATCTTA
					CCTCTAGAAT
31151	CTGAAGGCAC	AGCCTATACA	AACGCTGTTG	GATTTATGCC	TAACCTATCA
					ATTGGATAGT
31201	GCTTATCCAA	AATCTCACGG	TAAAACTGCC	AAAAGTAACA	TTGTCAGTCA
	CGAATAGGTT	TTAGAGTGCC	ATTTTGACGG	TTTTCATTGT	AACAGTCAGT

Figure 27 AG

31251	-			 ACCATTACAC TGGTAATGTG
31301				 ATACTCTATG TATGAGATAC
31351				 AAATATTTGC TTTATAAACG
31401				AGAATCGTTT TCTTAGCAAA
31451			TATTTTTCAA ATAAAAAGTT	 TTTCAAGTCA AAAGTTCAGT
31501			CCCCACCACC GGGGTGGTGG	 
31551			AGAACCCTAG TCTTGGGATC	
31601			AGTCCTTTCT TCAGGAAAGA	 
31651			ACATATTCTT TGTATAAGAA	 
31701			TCATCAGTGA AGTAGTCACT	
31751		· · ·	GCTGTCCAGC CGACAGGTCG	 
31801			CGGGCGCCGCT	
31851			TGCATCAGGA ACGTAGTCCT	 
31901			GCGCCGCCGC	 
31951	CATGGCAGTG GTACCGTCAC		CGATGATTCG GCTACTAAGC	 
32001	GCCTTGTCCT CGGAACAGGA		CAGCGCACCC GTCGCGTGGG	
32051	CAGTAACTGC GTCATTGACG			
32101	GGCGCTGTAT CCGCGACATA			 
	CATACCACAA			

Figure 27 AH

32251				ATCCACCACC TAGGTGGTGG	
32301				ACTGCAGGGA TGACGTCCCT	
32351				TAACCATGGA ATTGGTACCT	
32401				GCACACGTGC CGTGTGCACG	
32451				CCATATCCCA GGTATAGGGT	
32501				CAGGGAAGAC GTCCCTTCTG	
32551				TTCGGGCAGC AAGCCCGTCG	
32601				CAAAAGGAGG GTTTTCCTCC	
32651				GATCGTGTTG CTAGCACAAC	
32701				ATTTCCTGAA TAAAGGACTT	
32751				CGGTCTCGCC GCCAGAGCGG	
32801				TCTCAAAGCA AGAGTTTCGT	
32851				CATGCGCCGC GTACGCGGCG	
32901				AGCCAACCTA TCGGTTGGAT	
32951	CTGCGAGTCA GACGCTCAGT	CACACGGGAG GTGTGCCCTC	GAGCGGGAAG CTCGCCCTTC	AGCTGGAAGA TCGACCTTCT	ACCATGTTTT TGGTACAAAA
33001	TTATTTTTTT AATAAAAAA			TCAAAATGAA AGTTTTACTT	
33051	GTGAACGCGC CACTTGCGCG	TCCCCTCCGG AGGGGAGGCC	TGGCGTGGTC ACCGCACCAG	AAACTCTACA TTTGAGATGT	GCCAAAGAAC CGGTTTCTTG
33101	AGATAATGGC TCTATTACCG	ATTTGTAAGA TAAACATTCT	TGTTGCACAA ACAACGTGTT	TGGCTTCCAA ACCGAAGGTT	AAGGCAAACG TTCCGTTTGC

Figure 27 AI

33201				CGCCAAATAA CGGGTTTATT	
33251				CCCGAATATT	
33301				: ACCTTCAGCC	
	TAACATTTTT	AGACGAGGTC	TCGCGGGAGG	TGGAAGTCGG	AGTTCGTCGC
33351				CAGACCTGTA GTCTGGACAT	
33401	AAGCGGAACA TTCGCCTTGT			CCGTAGGTCC GGCATCCAGG	
33451				GGACCAGCGC CCTGGTCGCG	
33501				CTGATTATGA GACTAATACT	
33551	•			GTAAGCTTGT CATTCGAACA	
33601				AATCAGGCAA TTAGTCCGTT	
33651				TGCAGATAAA ACGTCTATTT	
33701				TTTTCTCTCA AAAAGAGAGT	
33751		-		ACAAAAAAAC TGTTTTTTTG	
33801				CCCTTATAAG GGGAATATTC	
33851				AACTGGTCAC TTGACCAGTG	
33901	AAGCACCACC TTCGTGGTGG			CGGAGTCATA GCCTCAGTAT	
33951	CGGTAAACAC GCCATTTGTG			TCAGTGCTAA AGTCACGATT	
34001	AAATAGCCCG TTTATCGGGC	-		CGTAGAGACA GCATCTCTGT	
34051	CCCCATAGGA GGGGTATCCT				



34151	ACATACAGCG TGTATGTCGC	CTTCCACAGC GAAGGTGTCG	GGCAGCCATA CCGTCGGTAT	ACAGTCAGCC TGTCAGTCGG	TTACCAGTAA AATGGTCATT
34201				GACACGGCAC CTGTGCCGTG	
34251				AGCGAGTATA TCGCTCATAT	
34301				AAACACCCAG TTTGTGGGTC	
34351	CGCTTGGATG	CGGGTCTTTG	CTTTCGGTTT	AAACCCACAA TTTGGGTGTT	GAAGGAGTTT
34401	AGCAGTGAAG	GCAAAAGGGT	GCAATGCAGT	CTTCCCATTT GAAGGGTAAA	ATTCTTTTGA
34451		TGTGTATGTT	CAATGAGGCG	GGATTTTGGA	TGCAGTGGGC
34501	CCCCGTTCCC GGGGCAAGGG	ACGCCCCGCG TGCGGGGCGC	CCACGTCACA GGTGCAGTGT	AACTCCACCC TTGAGGTGGG	GGAGTAATAG
		•			PacI
34551				TATTGATGAT ATAACTACTA	
34601				CCTTCCCCAT GGAAGGGGTA	
34651				TTGCAGGCCA AACGTCCGGT	
34701				TCAAGGCCAG AGTTCCGGTC	
34751				TTTTCCATAG AAAAGGTATC	
		TAGTGTTTTT	AGCTGCGAGT	TCAGTCTCCA	CCGCTTTGGG
		ATTTCTÄTGG	TCCGCAAAGG	GGGACCTTCG	AGGGAGCACG
		AGGCTGGGAC	GGCGAATGGC	CTATGGACAG	GCGGAAAGAG
34951	CCTTCGGGAA GGAAGCCCTT	GCGTGGCGCT CGCACCGCGA	TTCTCATAGC AAGAGTATCG	TCACGCTGTA AGTGCGACAT	GGTATCTCAG CCATAGAGTC
35001	TTCGGTGTAG AAGCCACATC	GTCGTTCGCT CAGCAAGCGA	CCAAGCTGGG GGTTCGACCC	CTGTGTGCAC GACACACGTG	GAACCCCCCG CTTGGGGGGC

Figure 27 AK

	AAGTCGGGCT	GCCGACGCGG	AATAGGCCAT	TGATAGCAGA	ACTCAGGTTG
35101	СССТАВСАС	ACCACTTATC	CCCACTCCCA	GCAGCCACTG	GTAACAGGAT
33101					CATTGTCCTA
	GGCCATTCTG	IGCIGMAING	COGIGACCGI	COICGGIGAC	CALIGICCIA
35151	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC
	ATCGTCTCGC	TCCATACATC	CGCCACGATG	TCTCAAGAAC	TTCACCACCG
35201	CTAACTACGG	CTACACTAGA	AGGACAGTAT	TTGGTATCTG	CGCTCTGCTG
	GATTGATGCC	GATGTGATCT	TCCTGTCATA	AACCATAGAC	GCGAGACGAC
	•				
35251	AAGCCAGTTA	CCTTCGGAAA	AAGAGTTGGT	AGCTCTTGAT	CCGGCAAACA
	TTCGGTCAAT	GGAAGCCTTT	TTCTCAACCA	TCGAGAACTA	GGCCGTTTGT
35301	AACCACCGCT	GGTAGCGGTG	GTTTTTTGT	TTGCAAGCAG	CAGATTACGC
*******					GTCTAATGCG
	110010000.	conredeeme	C. L.	.2.001.0010	0.01.211000
35351	GCAGAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC	TACGGGGTCT
	CGTCTTTTTT	TCCTAGAGTT	CTTCTAGGAA	ACTAGAAAAG	ATGCCCCAGA
35401	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG	TCATGAGATT
	CTGCGAGTCA	CCTTGCTTTT	GAGTGCAATT	CCCTAAAACC	AGTACTCTAA
35451	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATCAATCT	AAAGTATATA
	TAGTTTTTCC	TAGAAGTGGA	TCTAGGAAAA	TTTAGTTAGA	TTTCATATAT
35501	<b>2010</b>	moomones as	CDD1 CC11 DC	CMM1 1 mc 1 Cm	C1 CCC1 CCT1
33301		TGGTCTGACA			
	ACTCATTTGA	ACCAGACTGT	CAATGGTTAC	GAATTAGTCA	CTCCGTGGAT
35551	TCTCAGCGAT	CTGTCTATTT	CGTTCATCCA	TAGTTGCCTG	ACTCCCCGTC
		GACAGATAAA			
35601	GTGTAGATAA	CTACGATACG	GGAGGGCTTA	CCATCTGGCC	CCAGTGCTGC
	CACATCTATT	GATGCTATGC	CCTCCCGAAT	GGTAGACCGG	GGTCACGACG
		•			
35651	AATGATACCG	CGAGACCCAC	GCTCACCGGC	TCCAGATTTA	TCAGCAATAA
	TTACTATGGC	GCTCTGGGTG	CGAGTGGCCG	AGGTCTAAAT	AGTCGTTATT
35701		CGGAAGGGCC			
	TGGTCGGTCG	GCCTTCCCGG	CTCGCGTCTT	CACCAGGACG	TTGAAATAGG
35751	GCCTCCATCC				
	CGGAGGTAGG	TCAGATAATT	AACAACGGCC	CTTCGATCTC	ATTCATCAAG
35801	GCCAGTTAAT	<u>እርተሞተር</u> ርርር እ	<b>ACCUTC</b> TTCC	САТТССТАСА	CCCATCCTCC
33001		TCAAACGCGT			
	COGICAATIA	1CAMACGCG1	TOCANCANCO	GIMCGAIGI	CCGIAGCACC
35851	TGTCACGCTC	GTCGTTTGGT	ATGGCTTCAT	TCAGCTCCGG	TTCCCAACGA
33031	ACAGTGCGAG				
	UND 1 UND 1	T.100.121.00N			
35901	TCAAGGCGAG	TTACATGATC	CCCCATGTTG	TGCAAAAAAG	CGGTTAGCTC
	AGTTCCGCTC				
35951	CTTCGGTCCT	CCGATCGTTG	TCAGAAGTAA	GTTGGCCGCA	GTGTTATCAC
	GAAGCCAGGA	GGCTAGCAAC	AGTCTTCATT	CAACCGGCGT	CACAATAGTG



36051	AGATGCTTTT	CTGTGACTGG	TGAGTACTCA ACTCATGAGT	ACCAAGTCAT TGGTTCAGTA	TCTGAGAATA AGACTCTTAT
36101				GGCGTCAACA	
36101	CACATACGCC	GCTGGCTCAA	CGAGAACGGG	CCGCAGTTGT	GCCCTATTAT
36151	CCGCGCCACA	TAGCAGAACT	TTAAAAGTGC	TCATCATTGG AGTAGTAACC	AAAACGTTCT TTTTGCAAGA
26201				CTGTTGAGAT	
36201	AGCCCCGCTT	TTGAGAGTTC	CTAGAATGGC	GACAACTCTA	GGTCAAGCTA
36251	GTAACCCACT	CGTGCACCCA	ACTGATCTTC	AGCATCTTTT TCGTAGAAAA	ACTTTCACCA
				AAAATGCCGC	
36301	CGCAAAGACC	CACTCGTTTT	TGTCCTTCCG	TTTTACGGCG	TTTTTTCCCT
36351	ATAAGGGCGA	CACGGAAATG	TTGAATACTC	ATACTCTTCC TATGAGAAGG	TTTTTCAATA
				CATGAGCGGA	
36401	AATAACTTCG	TAAATAGTCC	CAATAACAGA	GTACTCGCCT	ATGTATAAAC
36451	AATGTATTTA	GAAAAATAAA	CAAATAGGGG	TTCCGCGCAC AAGGCGCGTG	ATTTCCCCGA
				ATTATCATGA	
36501	AAAGTGCCAC TTTCACGGTG	GACTGCAGAT	TCTTTGGTAA	TAATAGTACT	GTAATTGGAT
36551	TAAAAATAGG	CGTATCACGA	GGCCCTTTCG	TCTTCAAGAA	TTGGATCCGA
	ATTTTTATCC		CCGGGAAAGC	AGAAGTTCTT	AACCIAGGCI
		PacI			
2002	* BBCBB * * BBC	A COMPA A TOPA A	UK UI UASI	· 3 4 1	

36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34) TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Ingure 27 AM

VIRUS (P5)	PLASMID	VIRUS (P.	21)
MRKAcEgag(E3*) MRKAcEgag(E3*) 1 Kb*ladder	pAd5MRKgagSPA(E3*) pAd5MRKmCMVgag(E3*)	MRKAd5gag(E3-) MRKAd5gagSPA(E3*)	, MRKAd5mCMVgag(E3+)

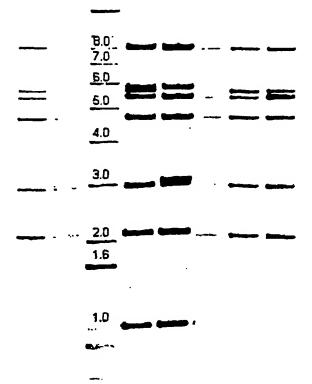


FIGURE 28

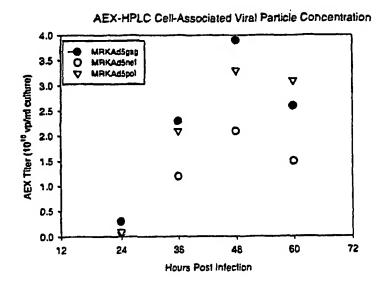


FIGURE 29A

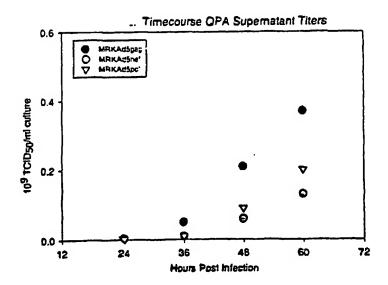


FIGURE 29B

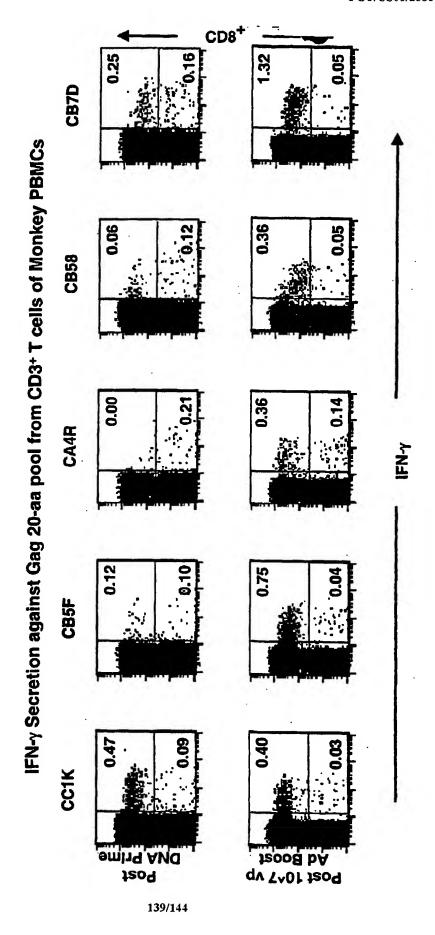
atg Met 1	gat Asp	gca Ala	atg Met	aag Lys 5	aga Arg	Gly	ctc Leu	tgc Cys	tgt Cys 10	gtg Val	ctg Leu	ctg Leu	ctg Leu	tgt Cys 15	gga Gly	48
			gtt Val 20													96
			agg Arg													144
			cag Gln													192
			ctg Leu													240
gtg Val	cac His	cag Gln	aag Lys	att Ile 85	gat Asp	gtg Val	aag Lys	gac Asp	acc Thr 90	aag Lys	gag Glu	gcc Ala	ctg Leu	gag Glu 95	aag Lys	288
			gag Glu 100													336
			Gly													384
cag Gln	aac Asn 130	ctc Leu	cag Gln	Gly	cag Gln	atg Met 135	gtg Val	cac His	cag Gln	gcc Ala	atc Ile 140	tcc Ser	ccc Pro	cgg Arg	acc	432
			tgg Trp													480
gtg Val	atc Ile	Pro	atg Met	ttc Phe 165	tct Ser	gcc Ala	ctg Leu	tct Ser	gag Glu 170	ggt Gly	gcc Ala	acc Thr	ccc Pro	cag Gln 175	gac Asp	528
ctg Leu	aac Asn	acc Thr	atg Met 180	ctg Leu	aac Asn	aca Thr	gtg Val	ggg Gly 185	Gly	cat His	cag Gln	gct Ala	gcc Ala 190	atg Met	cag Gln	576
atg Met	ctg Leu	aag Lys 195	gag Glu	acc Thr	atc Ile	aat Asn	gag Glu 200	Glu	gct Ala	gct Ala	gag Glu	tgg Trp 205	gac Asp	agg	ctg Leu	624
cat His	cct Pro 210	gtg Val	cac His	gct Ala	Gly	Pro 215	att Ile	gcc Ala	ccc Pro	Gly	cag Gln 220	atg Met	agg Arg	gag Glu	ccc Pro	672
agg Arg 225	G1y ggc	tct Ser	gac Asp	att Ile	gct Ala 230	ggc Gly	acc Thr	acc Thr	tcc Ser	acc Thr 235	ctc Leu	cag Gln	gag Glu	cag Gln	att Ile 240	720
ggc Gly	tgg Trp	atg Met	acc Thr	aac Asn 245	aac Asn	ecc Pro	ecc Pro	atc Ile	cct Pro 250	gtg Val	GJA BBB	gaa Glu	atc Ile	tac Tyr 255	aag Lys	768

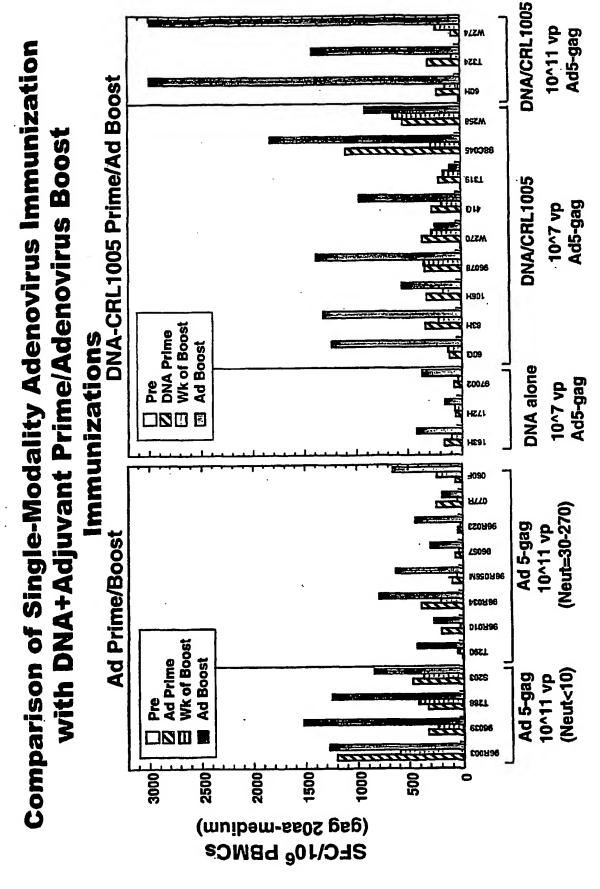
Figure 30'A"

agg Arg	tgg Trp	atc Ile	atc Ile 260	ctg Leu	ggc	ctg Leu	aac Asn	aag Lys 265	att Ile	gtg Val	agg Arg	atg Met	tac Tyr 270	tcc Ser	ccc Pro	816
acc	tcc Ser	atc Ile 275	ctg Leu	gac Asp	atc Ile	agg Arg	cag Gln 280	ggc Gly	ccc Pro	aag Lys	gag Glu	ecc Pro 285	ttc Phe	agg Arg	gac Asp	864
													gcc Ala			912
													aat Asn			960
													gcc			1008
													ggt Gly 350			1056
gcc Ala	agg Arg	gtg Val 355	ctg Leu	gct Ala	gag Glu	gcc Ala	atg Met 360	tcc Ser	cag Gln	gtg Val	acc Thr	aac Asn 365	tcc Ser	gcc Ala	acc Thr	1104
													aca Thr			1152
													tgt Cys			1200
													cac His			1248
													atc Ile 430			1296
													cct Pro			1344
													aag Lys			1392
													Pro			1440
tcc Ser	ctg Leu	agg Arg	tcc Ser	ctg Leu 485	ttt Phe	ggc Gly	aac Asn	gac Asp	ccc Pro 490	tcc Ser	tcc Ser	cag Gln	taa	(SI)	D NO:36) D NO:37)	1482

Figure 30 B

Figure 31





#### FIGURE 33A

ATGGGTGCTA	GGGCTTCTGT	GCTGTCTGGT	GGTGAGCTGG	ACAAGTGGGA	GAAGATCAGG
CTGAGGCCTG	GTGGCAAGAA	GAAGTACAAG	CTAAAGCACA	TTGTGTGGGC	CTCCAGGGAG
CTGGAGAGGT	TTGCTGTGAA	CCCTGGCCTG	CTGGAGACCT	CTGAGGGGTG	CAGGCAGATC
CTGGGCCAGC	TCCAGCCCTC	CCTGCAAACA	GGCTCTGAGG	AGCTGAGGTC	CCTGTACAAC
ACAGTGGCTA	CCCTGTACTG	TGTGCACCAG	AAGATTGATG	TGAAGGACAC	CAAGGAGGCC
CTGGAGAAGA	TTGAGGAGGA	GCAGAACAAG	TCCAAGAAGA	AGGCCCAGCA	GGCTGCTGCT
GGCACAGGCA	ACTCCAGCCA	GGTGTCCCAG	AACTACCCCA	TTGTGCAGAA	CCTCCAGGGC
CAGATGGTGC	ACCAGGCCAT	CTCCCCCGG	ACCCTGAATG	CCTGGGTGAA	GGTGGTGGAG
GAGAAGGCCT	TCTCCCCTGA	GGTGATCCCC	ATGTTCTCTG	CCCTGTCTGA	GGGTGCCACC
CCCCAGGACC	TGAACACCAT	GCTGAACACA	CTCCCCCCC	ATCAGGCTGC	CATGCAGATG
CTGAAGGAGA	CCATCAATGA	GGAGGCTGCT	GAGTGGGACA	GGCTGCATCC	TGTGCACGCT
GGCCCCATTG	CCCCCGGCCA	GATGAGGGAG	CCCAGGGGCT	CTGACATTGC	TGGCACCACC
TCCACCCTCC	AGGAGCAGAT	TGGCTGGATG	ACCAACAACC	CCCCATCCC	TGTGGGGGAA
ATCTACAAGA	GGTGGATCAT	CCTGGGCCTG	AACAAGATTG	TGAGGATGTA	CTCCCCCACC
TCCATCCTGG	ACATCAGGCA	GGGCCCCAAG	GAGCCCTTCA	GGGACTATGT	GGACAGGTTC
TACAAGACCC	TGAGGGCTGA	GCAGGCCTCC	CAGGAGGTGA	AGAACTGGAT	GACAGAGACC
CTGCTGGTGC	AGAATGCCAA	CCCTGACTGC	AAGACCATCC	TGAAGGCCCT	GGGCCCTGCT
GCCACCCTGG	AGGAGATGAT	GACAGCCTGC	CAGGGGGTGG	GGGCCCTGG	TCACAAGGCC
AGGGTGCTGG	CTGAGGCCAT	GTCCCAGGTG	ACCAACTCCG	CCACCATCAT	GATGCAGAGG
GGCAACTTCA	GGAACCAGAG	GAAGACAGTG	AAGTGCTTCA	ACTGTGGCAA	GGTGGGCCAC
ATTGCCAAGA	ACTGTAGGGC	CCCCAGGAAG	AAGGGCTGCT	GGAAGTGTGG	CAAGGAGGC
CACCAGATGA	AGGACTGCAA	TGAGAGGCAG	GCCAACTTCC	TGGGCAAAAT	CTGGCCCTCC
CACAAGGGCA	GGCCTGGCAA	CTTCCTCCAG	TCCAGGCCTG	AGCCCACAGC	CCCTCCCGAG
GAGTCCTTCA	GGTTTGGGGA	GGAGAAGACC	ACCCCCAGCC	AGAAGCAGGA	GCCCATTGAC
AAGGAGCTGT	ACCCCCTGGC	CTCCCTGAGG	TCCCTGTTTG	GCAACGACCC	CTCCTCCCAG
ATGGCTCCCA	TCTCCCCCAT	TGAGACTGTG	CCTGTGAAGC	TGAAGCCTGG	CATGGATGGC
	AGCAGTGGCC				
	AGAAGGAGGG				
	CCATCAAGAA				
	AGAGGACCCA				
	AGAAGAAGTC				
	AGGACTTCAG				
	TCAGGTACCA				
	CCTCCATGAC				
	AGTACATGGC				
	TTGAGGAGCT				
	AGAAGGAGCC				
	AGCCCATTGT				
	GCAAGCTGAA				
	TGCTGAGGGG				
GCTGAGCTGG	AGCTGGCTGA	GAACAGGGAG	ATCCTGAAGG	AGCCTGTGCA	TGGGGTGTAC

## FIGURE 33B

		03.mm00m03.0	*********	ACCCCCACCC	CCACTGGACC
	CCAAGGACCT				
	ACCAGGAGCC				
	CCAATGATGT				
	TCTGGGGCAA				
	GGACTGAGTA				
	TGGTGAAGCT				
ACCTTCTATG	TGGCTGGGGC	TGCCAACAGG	GAGACCAAGC	TGGGCAAGGC	TGGCTATGTG
ACCAACAGGG	GCAGGCAGAA	GGTGGTGACC	CTGACTGACA	CCACCAACCA	GAAGACTGCC
CTCCAGGCCA	TCTACCTGGC	CCTCCAGGAC	TCTGGCCTGG	AGGTGAACAT	TGTGACTGCC
TCCCAGTATG	CCCTGGGCAT	CATCCAGGCC	CAGCCTGATC	AGTCTGAGTC	TGAGCTGGTG
AACCAGATCA	TTGAGCAGCT	GATCAAGAAG	GAGAAGGTGT	ACCTGGCCTG	GGTGCCTGCC
CACAAGGGCA	TTGGGGGCAA	TGAGCAGGTG	GACAAGCTGG	TGTCTGCTGG	CATCAGGAAG
GTGCTGTTCC	TGGATGGCAT	TGACAAGGCC	CAGGATGAGC	ATGAGAAGTA	CCACTCCAAC
TGGAGGGCTA	TGGCCTCTGA	CTTCAACCTG	CCCCTGTGG	TGGCTAAGGA	GATTGTGGCC
	AGTGCCAGCT				
	AGCTGGCCTG				
GTGGCCTCCG	GCTACATTGA	GGCTGAGGTG	ATCCCTGCTG	AGACAGGCCA	GGAGACTGCC
TACTTCCTGC	TGAAGCTGGC	TGGCAGGTGG	CCTGTGAAGA	CCATCCACAC	TGCCAATGGC
TCCAACTTCA	CTGGGGCCAC	AGTGAGGGCT	GCCTGCTGGT	GGGCTGGCAT	CAAGCAGGAG
TTTGGCATCC	CCTACAACCC	CCAGTCCCAG	GGGGTGGTGG	CCTCCATGAA	CAAGGAGCTG
AAGAAGATCA	TTGGGCAGGT	GAGGGACCAG	GCTGAGCACC	TGAAGACAGC	TGTGCAGATG
GCTGTGTTCA	TCCACAACTT	CAAGAGGAAG	GGGGGCATCG	GGGGCTACTC	CGCTGGGGAG
AGGATTGTGG	ACATCATTGC	CACAGACATC	CAGACCAAGG	AGCTCCAGAA	GCAGATCACC
AAGATCCAGA	ACTTCAGGGT	GTACTACAGG	GACTCCAGGA	ACCCCCTGTG	GAAGGCCCT
GCCAAGCTGC	TGTGGAAGGG	GGAGGGGGCT	GTGGTGATCC	AGGACAACTC	TGACATCAAG
					GGCTGGGGAT
GACTGTGTGG	CCTCCAGGCA	GGATGAGGAC	TAA		
SEQ ID NO:					

#### FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

#### FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Jle Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp SEQ ID NO: 39

International application No.

PCT/US01/28861

IPC(7) US CL According to	SSIFICATION OF SUBJECT MATTER  : C12N 15/86  : 435/456  o International Patent Classification (IPC) or to both	national classification and IPC					
	DS SEARCHED	<del></del>					
	ocumentation searched (classification system followed 124/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173						
Documentati	ion searched other than minimum documentation to the	ne extent that such documents are include	d in the fields searched				
	ata base consulted during the international search (na continuation Sheet	me of data base and, where practicable, s	earch terms used)				
	UMENTS CONSIDERED TO BE RELEVANT	· · · · · · · · · · · · · · · · · · ·					
Category *	Citation of document, with indication, where a	ppropriate, of the relevant passages	Relevant to claim No.				
X	WO 96/39178 (ERTL et al.) 12 December 1996 (1)	2.12.1996), see page 5, 6,10, 12, 13	1-3, 8-11, 18				
Y	and claims 1 and 5.		4, 5, 13-17, 29-32, 34, 35, 37				
X	US 6,019,978 A (ERTL et al.) 1 February 2000,(0	1/02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18				
Y			4, 5, 13-17, 29-32, 34, 35, 37				
X,P	US 6,287,571 8 (ERTL et al.) 11 September 200 and claim 1.	01 (11/09/2001), see columns 2, 7, 8	1, 9, 18				
x	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/	1997), see examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18				
Ÿ			4,5,13-17, 29-32, 34, 35, 37				
Y	WANG et al. The use of an E1-deleted, replication expressing the rabies virus glycoprotein for early v Journal of Virology (March 1997) Vol. 71, No. 5,	accination of mice against rables virus.	1-3, 9-11, 13-18				
Purther	documents are listed in the continuation of Box C.	See patent family annex.					
• S	pecial entegories of cited documents:	"T" ister document published after the in priority date and not in conflict with					
	defining the general state of the art which is not considered to ticular relevance	understand the principle or theory us	derlying the invention				
"E" earlier ap date	plication or patent published on or after the international filing	"X" document of particular relevance; the considered novel or cannot be considered novel or step when the document is taken alor	tered to involve an inventive				
to establi	to establish the publication date of another citation or other special reason considered to involve an inventive step when the document is combined with one or more other such documents, such						
"O" document	referring to an oral disclosure, use, exhibition or other means	combination being obvious to a personal combination being obvious to a personal combination of the same patent					
	t published prior to the international filing date but later than the						
Date of the a	ctual completion of the international search 2002 (06.02.2002)	Date of mailing of the international sea	rch report				
	ailing address of the ISA/US	Authorized officer	1,10				
Cour	missioner of Patents and Trademarks PCT	Ulrike Winkler, Ph.D.	weeking for				
Was	hington, D.C. 20231	,	$\Gamma$				
Lacsumie No	o. (703)305-3230	Telephone No. 703-308-0196					

Form PCT/ISA/210 (second sheet) (July 1998)

### International application No.

### INTERNATIONAL SEARCH REPORT PCT/USO

Category •	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
<b>Y</b>	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29-32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29-32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
<b>Y</b> .,	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine, Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9
	,	
	·	
i		

International application No.

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Claim Nos.:     because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet
<ol> <li>As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.</li> <li>As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.</li> <li>As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:</li> </ol>
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37
Remark on Protest  The additional search fees were accompanied by the applicant's protest.  No protest accompanied the payment of additional search fees.

International application No.

PCT/US01/28861

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group	Claims	
1	1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Gag protein</u> (SEQ ID NO: 29) inserted in the <u>parallel orientation of E1</u> . In addition the vector contains a promoter and a polyadenylation signal.
2	6, 7, 36	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).
3	12, 33	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta EI$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of E1.
4	19-23, 38-42	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.
5	24, 27, 28, 43, 46, 47	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.
6	25, 26, 44, 45	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
7	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the parallel orientation of E1.
8	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AEI</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in the parallel orientation of E1.
9	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AEI</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in the parallel orientation of E1.
10	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E_1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the antiparallel orientation of E1.
11	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.
12	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.
13	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$

International application No.

		and ΔΕ3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AEI</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of Ε1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta EI$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type

International application No.

PCT/US01/28861

		and the state of t
-		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-pol fusion and one expressing gag.
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing gag-pol fusion and one expressing nef.
38	86e, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-gag fusion and one expressing pol.
39	86f, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein nef and gog are expressed individually from one vector.
45	861, 88, 89	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed as a fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed as a fusion protein from one vector.
48	860, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed as a fusion protein from one vector.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. End et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

International application No.

PCT/US01/28861

The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

#### (19) World Intellectual Property Organization International Bureau



## 

#### (43) International Publication Date 21 March 2002 (21.03.2002)

#### PCT

#### (10) International Publication Number WO 02/22080 A3

C12N 15/86 (51) International Patent Classification7:

(21) International Application Number: PCT/US01/28861

(22) International Filing Date:

14 September 2001 (14.09.2001)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data: 60/233.180 15 September 2000 (15.09.2000)

(71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]: 126 East Lincoln Avenue. Rahway. NJ 07065-0907 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): EMINI, Emilio, A. [US/US]: 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). YOUIL, Rima [AU/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). BETT, Andrew, J. [CA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CHEN, Ling [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). KASLOW, David, C. [US/US]: 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US), SHIVER, John, W. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US), TONER, Timothy, J. [US/US]: 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CASIMIRO, Daniel, R. [PH/US]: 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(74) Common Representative: MERCK & CO., INC.: 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(81) Designated States (national): AE. AG. AL. AM. AT. AU. AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS. LT. LU. LV, MA. MD. MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL. TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA. ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ. TZ, UG, ZW). Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CJ, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD. TG).

#### Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report: 2 May 2002

For two-letter codes and other abbreviations, refer to the "Guidunce Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIVI-GAG. POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV I- Gag. Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-I Gag, encoding codon optimized HIV-I Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated). HIV-1 Net and derivatives of optimized HIV-1 Net, including net mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

International application No.

IPC(7)	SIFICATION OF SUBJECT MATTER : C12N 15/86 : 435/456	otional classificati	on and IPC				
According to B. FIEL	International Patent Classification (IPC) or to both m DS SEARCHED	idonai ciassificati	on and it c				
Minimum doo U.S.: 42	cumentation searched (classification system followed 24/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3		30, 330/23.72,				
	on searched other than minimum documentation to the						
Electronic da Please See Co	ta base consulted during the international search (namontimuation Sheet	e of data base and	l, where practicable, s	earch terms used)			
C. DOC	UMENTS CONSIDERED TO BE RELEVANT						
Category *	Citation of document, with indication, where ap	propriate, of the r	elevant passages	Relevant to claim No.			
X  Y	WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6,10, 12, 13						
x 	US 6,019,978 A (ERTL et al.) 1 February 2000 (01.	/02/2000), see col	umns 2, 7 and 8.	1-3, 8-11, 18  4, 5, 13-17, 29, 30,			
X,P	32, 34, 35, 37						
Х  Y	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1	997), see example	es 1, 2, 25 and 26.	1-3, 8, 9-11, 18 			
Y	WANG et al. The use of an E1-deleted, replication expressing the rabies virus glycoprotein for early value of Virology (March 1997) Vol. 71, No. 5, p.	ccination of mice	irus recombinant against rabics virus.	34, 35, 37 1-3, 9-11, 13-18			
Purthe	r documents are listed in the continuation of Box C.	See pat	ent family annex.				
*A" documen	special categories of cited documents: t defining the general state of the art which is not considered to be	date and		emational filing date or priority cation but cited to understand the ention			
"E" carlier a	ular relevance pplication or patent published on or after the international filing date	consider	nt of particular relevance; the ed novel or cannot be conside a document is taken alone	claimed invention cannot be red to involve an inventive step			
establish specified		consider	nt of particular relevance; the ed to involve an inventive ste d with one or more other sue vious to a person skilled in the	p when the document is h documents, such combination			
"P" document priority	it referring to an oral disclosure, use, exhibition or other means it published prior to the international filing date but later than the date claimed	"&" docume	nt member of the same patent	family			
	actual completion of the international search 2002 (06.02.2002)	Date of mailing	of the international sea	arch report			
Name and n	nailing address of the ISA/US minissioner of Patents and Trademarks x PCT	Authorized offic Ulrike Winkler	3/1/1/	Collens)-la			
	sshington, D.C. 20231 (O. (703)305-3230	Telephone No.	703-308-0196	Treem -			
1	SA/210 (second sheet) (July 1998)						

International application No.

PCT/US01/28861

# INTERNATIONAL SEARCH REPORT

ategory •	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29, 30, 32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29, 30, 32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in maximalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	. 16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9
	·	

International application No.

PCT/US01/28861

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)				
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:				
1. Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:				
2. Claim Nos.: 31 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically: This claim could not be searched because applicant did not provide a CRF.				
3. Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).				
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)				
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet				
As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.				
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite				
payment of any additional fee.  3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:				
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37				
Remark on Protest The additional search fees were accompanied by the applicant's protest.				
No protest accompanied the payment of additional search fees.				

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)

International application No.

PCT/US01/28861

The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

#### Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

International application No.

	1	and ΔE3, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1)
		inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of <u>AE1</u>
	1	and ΔE3, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5)
		inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7)
		inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant
		adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response
17	02, 03, 00	to HIV Pol protein with the recombinant adenoviral particle.
••		The claim is directed to a method of generating a cellular mediated immune response
18	63, 64	to HIV Pol protein with the recombinant adenoviral particle in addition to
		to HIV Poi protein with the recombinant adenoviral particle in addition to
		administering a DNA plasmid vaccine.
19	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in the parallel orientation of Et.
20	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
	Ì	inserted in the parallel orientation of E1.
21	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type
	/51 /5	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
	1	inserted in the parallel orientation of E1.
22	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
22		ΔE1, the vector contains the cis-acting packaging sequence of the wild type
	73, 75	adenovirus genome, and a gene which encodes an HIV Nei protein (SEQ ID NO: 15)
	i	
		inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ ,
		the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in
		the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ ,
		the vector contains the cis-acting packaging sequence of the wild type adenovirus
	1	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in
		the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta EI$ ,
	1	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in
		the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ .
20	1"	the vector contains the cis-acting packaging sequence of the wild type adenovirus
	1	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in
	-	the antiparallel orientation of E1.
	<del> </del>	
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta EI$
		and ΔE3, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in E1.
	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
28	1	and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type
28		· · · · · · · · · · · · · · · · · · ·
28		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
28		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
	7.4	inserted in E1
28	74	

International application No.

		and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type
	İ	adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1)
		inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta EI$
14	33	and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5)
	j	inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
13	1 33	and AE3, the vector contains the cis-acting packaging sequence of the wild type
	İ	adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7)
		inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant
10	37-01	adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response
17	02, 05, 00	to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response
10	05, 04	to HIV Pol protein with the recombinant adenoviral particle in addition to
	:	administering a DNA plasmid vaccine.
10	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
19	73, 75	AE1 the vector contains the cis-acting packaging sequence of the wild type
	13, 13	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in the parallel orientation of E1.
20	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
20	73, 75	AF1 the vector contains the cis-acting packaging sequence of the wild type
	13, 73	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
	i	inserted in the parallel orientation of E1.
21	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	AE1 the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
		inserted in the parallel orientation of E1.
22	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
	_	inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ .
	-	the vector contains the cis-acting packaging sequence of the wild type adenovirus
	i i	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in
		the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ ,
		the vector contains the cis-acting packaging sequence of the wild type adenovirus
	İ	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in
		the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E I$ .
	ļ	the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in
		genome, and a gene which encodes an HIV Net protein (32Q 15 140. 15) has red at
		the antiparallel orientation of E1.  The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ .
26	71	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in
		the antiparallel orientation of E1.
	<del></del>	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
27	74	and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type
Į.		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
]		inserted in E1.
100	<del>  74</del>	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
28	74	and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type
	1	and AE3, the vector contains the cis-acting packaging sequence of the ward speakaging sequence
Į	ł	inserted in E1.
-	<del>-  </del>	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
29	74	and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type
<u> </u>		tota DDS , the roots sometimes are an angle of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party of the party

International application No.

PCT/US01/28861

	T	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
		inserted in E1.
	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta EI$
30	/*	l and A E3 the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
•		inserted in E1.
	<del> </del>	The claims are directed to a method of making and harvesting of a recombinant
31	76-80	adenoviral particle that contains a gene encoding an HIV Nef protein.
	04.05	The claims are directed to a method of generating a cellular mediated immune
32	81, 84, 85	response to HIV Nef with the recombinant adenoviral particle.
	<u> </u>	The claims are directed to a method of generating a cellular mediated immune
33	82, 83	response to HIV Nef with the recombinant adenoviral particle in addition to
	ì	response to HIV Net with the recombinant accnownal parties in additional to
		administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed
,		from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
33		from one individual vectors.
	86c, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
36	800, 00	from two individual vectors, one expressing nef-pol fusion and one expressing gag.
	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
37	800, 87, 80	from two individual vectors, one expressing gag-pol fusion and one expressing nef.
	- 24 99	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
38	86c, 88	from two individual vectors, one expressing nef-gag fusion and one expressing pot.
		The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
39	86f, 88	from a single vectors as a fusion protein.
l		The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
40	86g, 88	from two individual vectors.
		The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
41	86h, 88, 89	The claims are drawn to a militivatent vaccine wherein gag and pot are expressed
''		individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
172		from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
43	3.	from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
444	Joan, Go	individually from one vector.
<del></del>	861, 88, 89	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
45	801, 80, 05	individually from one vector.
<u> </u>	86m, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed as a
46	80m, 80	fision protein from one vector.
		The claims are drawn to a multivalent vaccine wherein pol and nef are expressed as a
47	86n, 88	fusion protein from one vector.
		The claims are drawn to a multivalent vaccine wherein nef and gag are expressed as a
48	86u, 88	The claims are grawn to a minimized in vaccule wherein hey and gub are expressed as a
L		fusion protein from one vector.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Erd et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

### REVISED VERSION

#### (19) World Intellectual Property Organization International Bureau





#### (43) International Publication Date 21 March 2002 (21.03.2002)

#### **PCT**

#### (10) International Publication Number WO 02/022080 A3

C12N 15/86 (51) International Patent Classification7:

(21) International Application Number: PCT/US01/28861

(22) International Filing Date:

14 September 2001 (14.09.2001)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/233,180

15 September 2000 (15.09.2000) US

(71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(72) Inventors; and

- (75) Inventors/Applicants (for US only): EMINI, Emilio, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). YOUIL, Rima [AU/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). BETT, Andrew, J. [CA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CHEN, Ling [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). KASLOW, David, C. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SHIVER, John, W. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). TONER, Timothy, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CASIMIRO, Daniel, R. [PH/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,

#### Published:

with international search report

(88) Date of publication of the international search report: 2 May 2002

Date of publication of the revised international search 16 January 2003 report:

(15) Information about Corrections:

see PCT Gazette No. 03/2003 of 16 January 2003, Sec-

**Previous Correction:** 

see PCT Gazette No. 30/2002 of 25 July 2002, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HTV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HTV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1- Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HTV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HTV-1 Pol is inactivated). HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.



International application No.

A. CLA IPC(7) US CL	SSIFICATION OF SUBJECT MATTER : C12N 15/86 : 435/456		
	o International Patent Classification (IPC) or to both	national classification and IPC	
Minimum d	ocumentation searched (classification system follower 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173	d by classification symbols) .3, 235.1, 320.1, 456; 530/23.72;	
Documentat	ion searched other than minimum documentation to the	ne extent that such documents are include	d in the fields searched
	ata base consulted during the international search (na Continuation Sheet	me of data base and, where practicable, a	search terms used)
	UMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where a	ppropriate, of the relevant passages	Relevant to claim No.
<b>x</b> 	WO 96/39178 (ERTL et al.) 12 December 1996 (1) and claims 1 and 5.	2.12.1996), see page 5, 6,10, 12, 13	1-3, 8-11, 18
Y	·		4, 5, 13-17, 29-32, 34, 35, 37
<u>x</u>	US 6,019,978 A (ERTL et al.) 1 February 2000,(0	1/02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18
Y			4, 5, 13-17, 29-32, 34, 35, 37
X,P	US 6,287,571 $\beta$ (ERTL et al.) 11 September 200 and claim 1.	01 (11/09/2001), see columns 2, 7, 8	1, 9, 18
<u>x</u>	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/	1997), see examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18
Y			4,5,13-17, 29-32, 34, 35, 37
Y	WANG et al. The use of an E1-deleted, replication expressing the rabies virus glycoprotein for early v Journal of Virology (March 1997) Vol. 71, No. 5,	accination of mice against rabies virus.	1-3, 9-11, 13-18
Furthe	documents are listed in the continuation of Box C.	See patent family annex.	L
	pecial categories of cited documents:	T" later document published after the in	stemptional filing data or
"A" documen	t defining the general state of the art which is not considered to	priority date and not in conflict with understand the principle or theory u	the application but cited to
"E" earlier ay	pilication or patent published on or after the international filling	"X" document of particular relevance; the considered novel or cannot be consisted when the document is taken alo	dered to involve an inventive
"L" documen to establ (as speci	t which may throw doubts on priority claim(s) or which is cited sh the publication date of another cliation or other special reason fied)	"Y"  document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
	t referring to an oral disclosure, use, exhibition or other means	"&" document member of the same paten	
*P* document	published prior to the international filing date but later than the		
Date of the	Date of the actual completion of the international search  Date of mailing of the international search report  19 AUG 2002		
	Name and mailing address of the ISA/US  Authorized officer		
Commissioner of Patents and Trademarks Box PCT  Ulrike Winkler, Ph.D.			alllens for
	hington, D.C. 20231 D. (703)305-3230	Telephone No. 703-308-0196	1)
Form PCT/IS	A/210 (second cheet) (July 1008)	· · · · · · · · · · · · · · · · · · ·	

### International application No.

PCT/US01/28861

### INTERNATIONAL SEARCH REPORT

lategory •	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
<b>Y</b>	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29-32
Y	PREVEC et al. Immine response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and thesus macaque monkeys. Journal of Acquired Immine Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29-32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
<b>Y</b>	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	_ 1,9
!		

International application No.

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)			
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:			
Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:			
Claim Nos.:  because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:			
3. Claim Nos.:  because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule  6.4(a).			
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)			
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet			
1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.  2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.  3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:			
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37			
Remark on Protest  The additional search fees were accompanied by the applicant's protest.  No protest accompanied the payment of additional search fees.			

International application No.

PCT/US01/28861

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group	Claims		
1	1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta EI$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29) inserted in the parallel orientation of E1. In addition the vector contains a promoter and a polyadenylation signal.	
2	6, 7, 36	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> and <u>AE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).	
3	12, 33	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of E1.	
4	19-23, 38-42	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.	
5	24, 27, 28, 43, 46, 47	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.	
6	25, 26, 44, 45	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.	
7	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AEI</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in the parallel orientation of E1.	
8	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in the parallel orientation of E1.	
9	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the parallel orientation of E1.	
10	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta EI$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the antiparallel orientation of E1.	
11	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.	
12	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.	
13	55	The claim is directed to an adenoviral vector that is at least partially deleted of AE1	

Form PCT/ISA/210 (second sheet) (July 1998)

International application No.

PCT/US01/28861

		and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEO ID NO: 1)
		inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5)
		inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and ΔΕ3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant
	10 10 10	adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to
19	67-70, 72,	administering a DNA plasmid vaccine.  The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	<u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.
20	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.
21	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	$\Delta$ E1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.
22	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ ,
		the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ ,
		the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ ,
		the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in
26	71	the antiparallel orientation of E1.  The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta B1$ ,
20	,,	the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in
27	74	the antiparallel orientation of E1.  The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta$ E1
_,		and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of AE1
		and $\Delta$ E3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E_1$
	1	and AE3, the vector contains the cis-acting packaging sequence of the wild type

Form PCT/ISA/210 (second sheet) (July 1998)

International application No.

PCT/US01/28861

		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
	İ	inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and AE3, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
		inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant
		adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune
		response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune
		response to HIV Nef with the recombinant adenoviral particle in addition to
	4	administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from two individual vectors, one expressing nef-pol fusion and one expressing gag.
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from two individual vectors, one expressing gag-pol fusion and one expressing nef.
38	86e, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from two individual vectors, one expressing nef-gag fusion and one expressing pol.
39	86f, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
		from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
	<del>                                     </del>	individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
40	06: 00.00	from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
44	061- 00	from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed individually from one vector.
45	861, 88, 89	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
45	001, 00, 09	individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed as
70	0011, 00	fusion protein from one vector.
47	86p. 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed as
••	5524, 55	fusion protein from one vector.
48	860, 88	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed as a
	1 000, 00	I am arrange and are are an a minimatering successive afficient und area for an explication as a

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Ertl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

International application No.

PCT/US01/28861

The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3: WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter